Capstone Project

Machine Learning Engineer Nanodegree

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Definition

Project Overview

Mass spectrometry is an analytical technique that ionizes chemical or biological material and measures mass-to-charge ratio (m/z) of ions (mass spectrum). In simple terms, a mass spectrum measures distribution of masses in a sample. Knowing mass to molecule [1] correspondence makes it possible to use mass spectrometry to annotate all peaks in a spectrum with molecules they can originate from.

Mass spectrometry imaging (MSI) is a technique used in mass spectrometry to visualize spatial distribution of masses (or molecules) in a sample. In MSI, a dataset can be represented as a 3-dimensional tensor ($N \times M \times K$). Where N and M are spatial dimensions (acquisition matrix) and K is a mass dimension (m/z bins). What is unusual about these images is that number of channels always exceeds spatial size of images. At the same time, the number of mass channels most of the time is excessive and can be reduced.

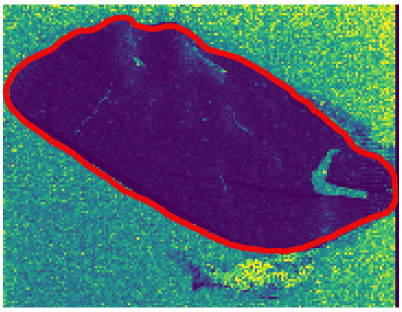
Pretty often during MSI acquisition, spectra are collected from a sample and its nearby area. Also some instruments are restricted to rectangular acquisition areas only. This leads to a problem of separation of signals (spectra) coming from the sample itself from background signals coming primarily from the nearby area.

European project METASPACE aims to provide information about spatial distribution of small molecules of biological origin (metabolites) in samples provided by users in the form of MSI datasets. Implementation of a method for distinguishing between molecules coming from sample and background molecules could greatly improve quality of MSI data annotation provided by METASPACE.

[1] Throughout the report we will be using the term *ion* instead of *molecule* because it describes the data used in the project more precisely.

Problem Statement

Figure 1: A slice of an MSI dataset for a specific m/z (or associated ion). The inner part of manually outlined region (in red) is a sample area, the outside area is off sample (or background). Here we have an example of a signal that mostly comes (more intense signals are yellow) from the off sample area and thus not particularly interesting to the user.



The METASPACE project currently hosts results for more than 3000 MSI datasets. The results are represented as a set of images corresponding to different ions. For a number of MSI datasets, it is possible to define off sample areas (or images with signal coming from off sample areas) using expert knowledge. This way, we can get the ground truth data for the problem in focus.

Originally, each MSI dataset is represented by a 3 dimensional tensor $N \times M \times K$ with mass measurements for each pixel, plus a binary mask of $N \times M$ shape (each pixel assigned one of two classes). It is also possible to represent each pixel data as an N dimensional vector. In this form, the data can be used to train a binary classification model to predict pixel classes (or off sample masks) for new MSI datasets.

It is important to mention that the data in its original form does not contain pixel labels (MSI dataset off sample masks). That is why it is a part of the project to label the data so that a supervised algorithm can be used.

Metrics

Model performance can be measured the same way as in any other binary classification task, using accuracy, AUC, F1 or precision and recall scores.

Analysis

Data Exploration

To solve the problem defined above, a subset of 50 MSI datasets was collected, one dataset per MSI experiment. So the target dataset contains data from multiple MSI datasets. Each MSI dataset contains several thousand measurements or mass spectra acquired on a regular two-dimensional grid. One spectrum is a one-dimensional vector with thousands elements. Each spectrum (or pixel) is located on the sample or off sample (background) area. So each pixel can be assigned one of the two classes (on/off sample).

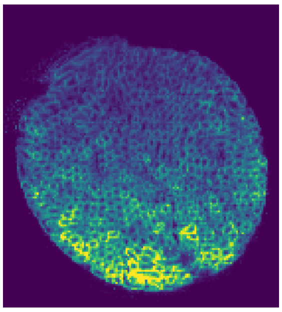


Figure 2: m/z (ion) image example

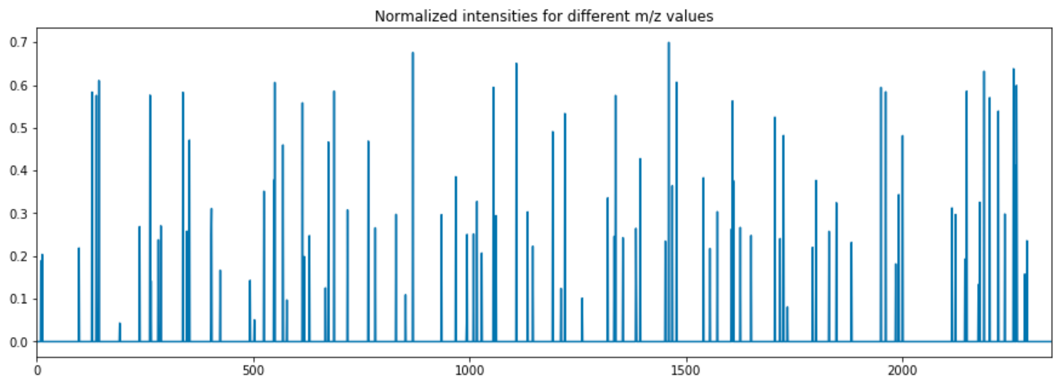
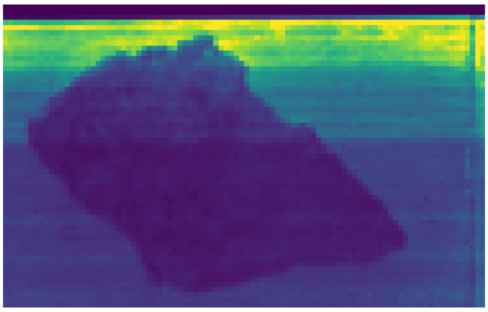


Figure 3: Single (normalized) spectrum example

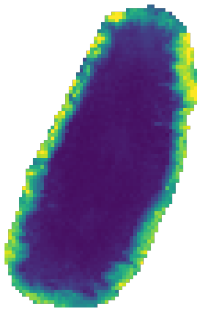
The MSI datasets can be aquired with different instruments in quite different conditions, biological samples can also be very diverse. Because of this, spectra (pixels) that have the same class but belong to different MSI datasets can be pretty unsimilar. Additional difficulties are caused by some MSI datasets being not rectangular but of arbitrary shape. Which makes off sample areas around sample in those datasets really small. The amount of signal for every MSI dataset can be quite different as well. This makes comparison of spectra between MSI datasets a challenging task.

Exploratory Visualization

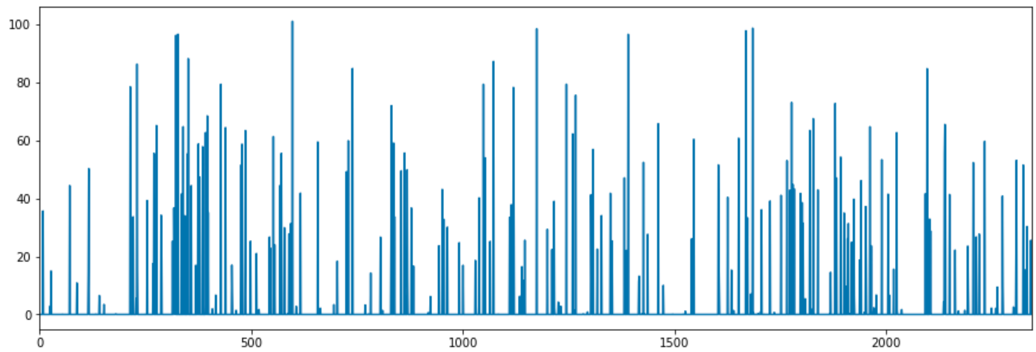
Figure 4: Two images above are slices of MSI datasets of regular and irregular shapes. It is pretty easy to see two distinct areas on the first image. On the second one, it is tough to locate the off sample area as it is represented only with a thin ring around the sample.



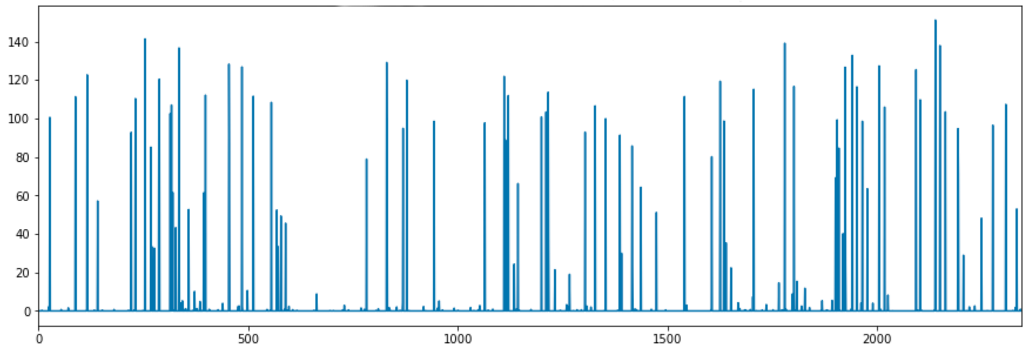
(a) Rectangular MSI dataset



(b) Irregular shape MSI dataset



(a) Sum of on sample spectra



(b) Sum of off sample spectra

Figure 5: The above two images show sum of spectra for two areas (on/off sample) of a MSI dataset. It is easy to see that there is a difference between these two spectra.

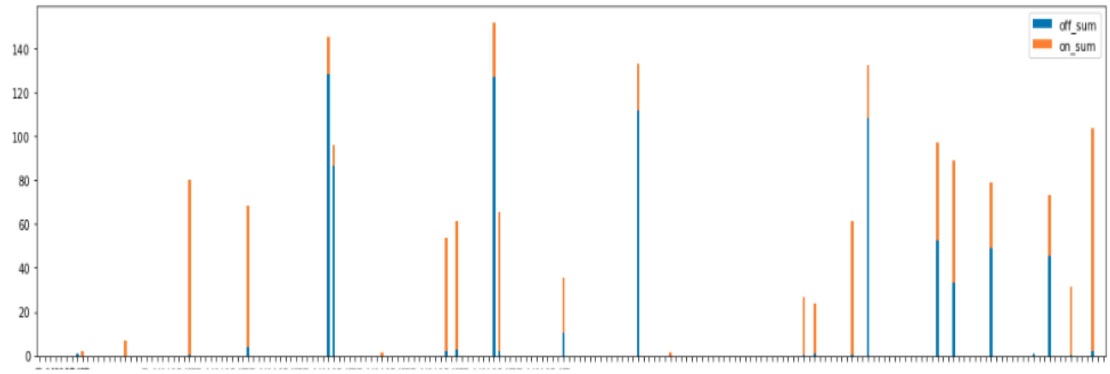


Figure 6: The stacked bars graph is a zoomed version of the above two spectra combined. On this graph, it is easier to see that some signals (at specific m/z) come mostly from the off sample while others from the on sample area.

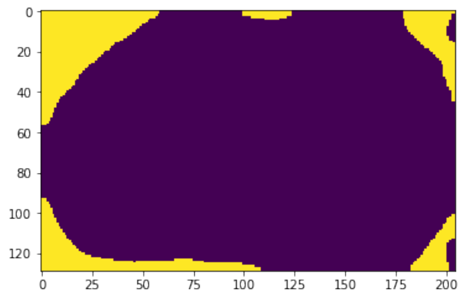
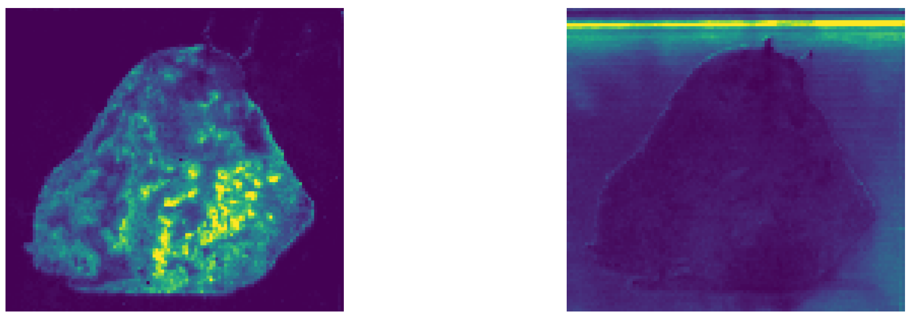


Figure 7: The example mask for a MSI dataset, the mask is in yellow. Each pixel has one of the two classes assigned to it. Correct prediction of such masks for all MSI datasets in focus is one way of solving the original problem. Once we have a mask image for a MSI dataset, it should be possible to combine it with every result image (ion image) from METASPACE for the dataset and decide if the image depicts the off sample or the on sample area.



(a) Ion image of the on sample area

(b) Ion image of the off sample area

Figure 8: Another approach would be to try to train a model that takes an ion image as input and classifies it as on/off sample one. Two images from the same MSI dataset, the left one depicts the on sample area, the right off sample.

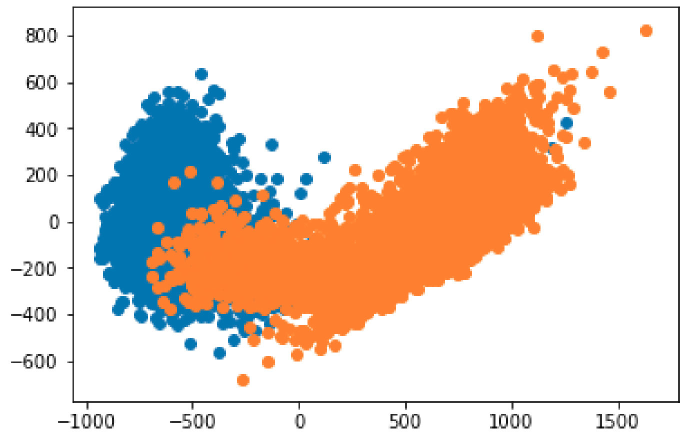


Figure 9: The PCA tranformation was applied to all pixels of a MSI dataset. Visualisation of the first two components. Pixels that belong to different classes have different colour. Here we can clearly see two groups that also have a substantial ovelapping.

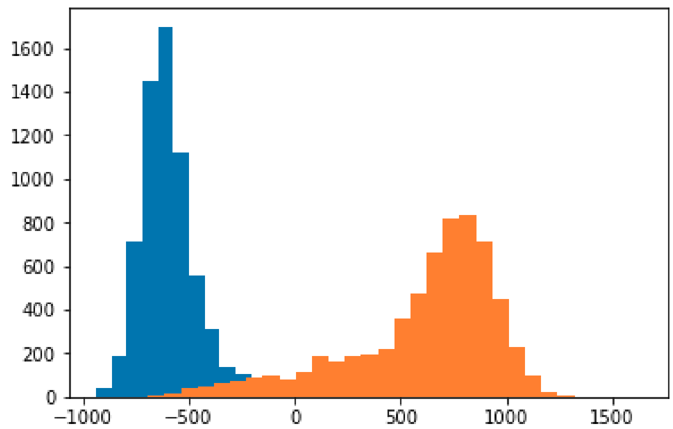


Figure 10: A histogram of the first principal component. It is easy to see that distributions of of component values are quite different for the classes. Unfortunately, not for all MSI datasets in focus it is possible to get such a nice binomial distribution. Another problem with this approach is in the fact that it is not known which class a particular distribution represents, on or off sample. The order of distribution peaks is different for each MSI dataset.

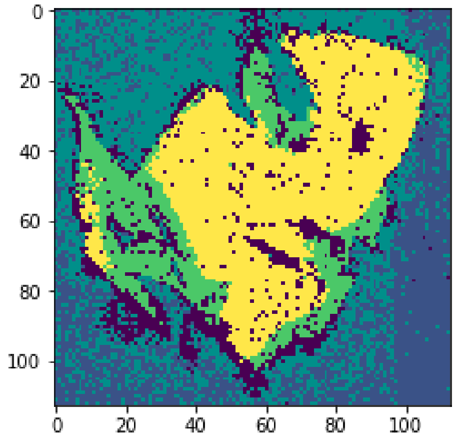


Figure 11: Another attempt to use unsupervised learning in order to separate pixels of two different classes. K-means clustering algorithm with $N = 5$ (N - number of clusters) was applied to an MSI dataset. The cluster numbers were assigned colours and mapped back to the original pixel locations. One can clearly see a nice groupping of pixels. Here the clusters assigned yellow, green and dark blue colours together represent on sample area.

Algorithms and Techniques

As mentioned before, the original problem has two versions:

- Prediction of an off sample mask and using this mask to split all ion images into two groups (on and off sample).
- Taking ion images as input and directly classifying them into two groups.

For the first version of the problem, it is easier to apply a machine learning technique as the off sample mask is simply all MSI dataset pixels with one of the two classes assigned to them. So the algorithms for independent classification of pixels were explored. The input vectors for pixel examples were intensities of different ions, an improved version of the pixel mass spectrum. In this form, any binary classification algorithm can be applied. Wide range of models from scikit-learn Python package have been applied. The most satisfying results were obtained with Random Forest, Naive Bayes Classifier and Fully Connected Neural Network.

The second step of mask application to ion images, that initially seemed easy, appeared to be quite challenging and achievement of good results was not possible. That is why the second version of the original problem was explored more thoroughly.

Since inputs were two dimensional single channel images, the available options for models were limited. At the moment, Convolutional Neural Networks (CNNs) dominate the field of image recognition and classification. [2] Python package Keras with TensorFlow as a backend was used as the easiest way to start experimenting with CNNs.

[2]"Review of Deep Learning Algorithms for Image Classification" `https://medium.com/comet-app/review-of-deep-learning-algorithms-for-image-classification-5fdbca4a05e2`

Benchmark

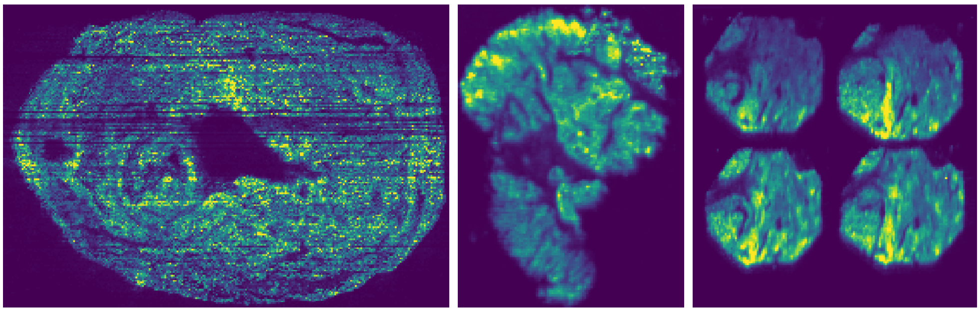
As a simple benchmark model, binary classifiers like Logistic Regression from scikit-learn were tested to predict individual classes of all pixels from the test MSI datasets. Classes of predicted labels were used to create off sample masks. Correlation of off sample masks and ion images from the test MSI datasets with thresholding was used to classify ion images into two classes (on or off sample).

The best models, Random Forest and Fully Connected Neural Network, combined with correlation postprocessing had similar performance and were used as the base benchmark model that achieved the following best results: $recall = 0.8$, $precision = 0.75$

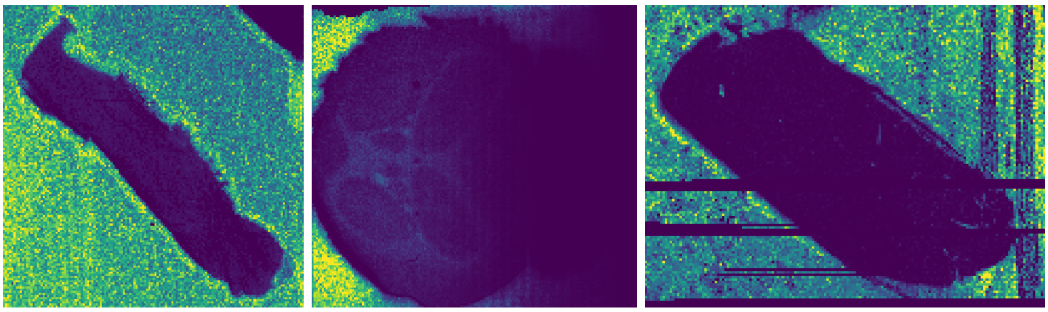
Methodology

Data Preprocessing

About one hundred MSI datasets from METASPACE were selected to be used in the final model training stage. Only MSI datasets of regular shape (rectangular) were used. Futher, using expert knowledge, all ion images for each MSI dataset were separated into two classes, on and off sample.



(a) On sample ion images



(b) Off sample ion images

Figure 12: Comparison of on sample (top) and off sample (bottom) ion images.

The final image distribution by classes:

- off sample - 7451
- on sample - 15665

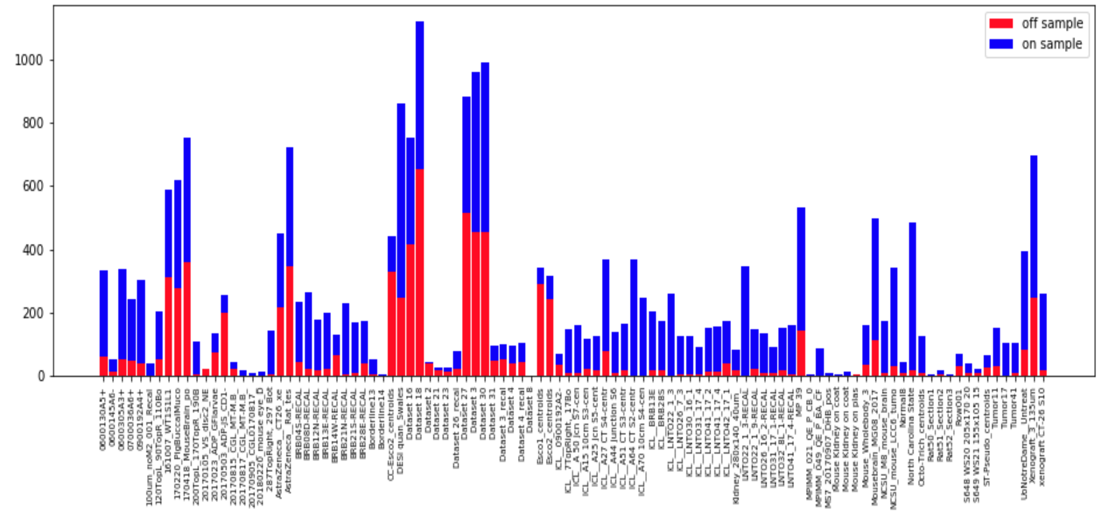


Figure 13: The final dataset appeared to be slightly unbalanced with approximately 30% of target class images. But inside image groups (MSI datasets), image classes can be much more imbalanced. In some cases, a group has just a few images of the target class. This fact definitely added complexity to the problem.

As the next step, all the ion images were scaled into square images of 56×56 size. These images, grouped by MSI dataset they belong to, were used as inputs to the model.

Implementation

A Convolutional Neural Network implemented in Keras with TensorFlow as a backend was used as the model of choice for image classification. During model development the following questions have been explored:

- General CNN architecture decisions. How many convolutional and dense layers the model should have. As image features to be learned were not too complicated and number of classes was low, the network did not need to be too deep. Experiments confirmed that just four convolutional and three dense layers were enough.
- Number of units for each layers, the shapes of convolutional filters, size of strides and padding type were optimized with aim to have several hundreds thousand parameters to learn. At the same time, it was important to avoid excessive growth of parameters at the first dense layer. Large number of total network parameters also leads to network overfitting to easily. [3]
- Types of activation functions. ReLU activation functions [4] proved to be effective and fast during backpropagation calculation. The problem of fading gradients affects them less than sigmoid or tahn functions.

[3]Generalization: Peril of Overfitting `https://developers.google.com/machine-learning/crash-course/generalization/peril-of-overfitting`

[4]Glorot, Xavier et al. "Deep Sparse Rectifier Neural Networks." AISTATS (2011)

- Number of max pooling layers was chosen to be half of convolutional layers number. Often in deep CNNs, only every second convolutional layer is followed by a max pooling layer. Here we followed the same rule.

- L2 regularization, use of dropout. [5] L2 regularization did not help to improve the model accuracy but made it more stable while training. Use of dropout had some positive effects on model accuracy before batch normalisation was introduced after every convolutional layer. After that dropout was removed even from the dense layers.

- Batch normalisation [6] was added after every convolutional layer and greatly improved model accuracy and robustness. Normalising layer weights internally after every batch helps to improve model training speed as well. After adding it to the model it was enough to train the model for a couple of epochs.

- Loss function choice. Cross-entropy is recommended as an effective loss function for image classification tasks. In our case, we used binary cross-entropy as the task has two classes.

- Optimization algorithm and its hyperparameters. Experiments with a few different optimization algorithms has been done but the most effective appeared to be Stochastic Gradient Descent with momentum. Its hyperparameters were chosen during number of experiments and were close to those usually recommened ($learning\_rate = 0.01$ and $momentum = 0.9$).

- Model complexity evaluation and running time estimation. While optimizing the model architecture it was important to keep the number of parameters high but not increase their number too much as it is known to be prone to overfitting. It was also important to keep running time under several minutes because otherwise it was not feasible to complete all experiments each of which included ten cross-validation runs with five repetitions for each cross-validation fold. More details below.

- Improving model robustness so that results do not change significantly between runs. From the very beginning, due to some imbalance in the data mentioned above, the model results were slightly different after every training cycle on the same data. Lots of approaches have been explored in order to mitigate this issue. But eventually, the most effective one was just to train five different models on the same data and average their predictions.

[5] Srivastava, Nitish et al. "Dropout: A Simple Way to Prevent Neural Networks from Overfitting." Journal of Machine Learning Research 15 (2014).

[6] Szegedy, Christian et al. "Batch Normalization: Accelerating Deep Network Training by Reducing Internal Covariate Shift." arXiv preprint arXiv:1502.03167 (2015).

```
_________________________________________________________________
Layer (type)                 Output Shape              Param #
=================================================================
conv2d_5 (Conv2D)            (None, 56, 56, 16)        160
_________________________________________________________________
batch_normalization_5 (Batch (None, 56, 56, 16)        64
_________________________________________________________________
activation_8 (Activation)    (None, 56, 56, 16)        0
_________________________________________________________________
conv2d_6 (Conv2D)            (None, 56, 56, 16)        2320
_________________________________________________________________
batch_normalization_6 (Batch (None, 56, 56, 16)        64
_________________________________________________________________
activation_9 (Activation)    (None, 56, 56, 16)        0
_________________________________________________________________
max_pooling2d_3 (MaxPooling2 (None, 18, 18, 16)        0
_________________________________________________________________
conv2d_7 (Conv2D)            (None, 18, 18, 32)        4640
_________________________________________________________________
batch_normalization_7 (Batch (None, 18, 18, 32)        128
_________________________________________________________________
activation_10 (Activation)   (None, 18, 18, 32)        0
_________________________________________________________________
conv2d_8 (Conv2D)            (None, 18, 18, 32)        9248
_________________________________________________________________
batch_normalization_8 (Batch (None, 18, 18, 32)        128
_________________________________________________________________
activation_11 (Activation)   (None, 18, 18, 32)        0
_________________________________________________________________
max_pooling2d_4 (MaxPooling2 (None, 6, 6, 32)          0
_________________________________________________________________
flatten_2 (Flatten)          (None, 1152)              0
_________________________________________________________________
dense_4 (Dense)              (None, 256)               295168
_________________________________________________________________
activation_12 (Activation)   (None, 256)               0
_________________________________________________________________
dense_5 (Dense)              (None, 256)               65792
_________________________________________________________________
activation_13 (Activation)   (None, 256)               0
_________________________________________________________________
dense_6 (Dense)              (None, 2)                 514
_________________________________________________________________
activation_14 (Activation)   (None, 2)                 0
=================================================================
Total params: 378,226
Trainable params: 378,034
Non-trainable params: 192
```

Figure 14: The final network architecture

Results

Model Evaluation and Validation

The final model had four convolutional layers followed by three dense layers. All convolutional layers had batch normalisation applied. Every second one was followed by a max pooling layer. All activation functions were ReLU except for the last layer where softmax was used. Binary cross-entropy was used as the loss function. Stochastic Gradient Descent with momentum was used as the optimization algorithm.

In order to evaluate robustness of the trained model, cross-validation technique was used. Because the dataset had internal groups of images, it was important to take this information into account when making cross-validation folds. Not following this approach resulted into model heavily overfitting due to information bleeding into the validation sets through similarities of the same group images presented in both train and validation sets. GroupKFold from scikit-learn Python package was used to implement correct cross-validation.

Another challenge to be addressed was model robustness. The model kept providing slightly different results on the validation set being trained on the same train set. This fact made model accuracy evaluation and hyperparameters optimization a more difficult task. All the attempts to add different types of regularization and improve optimization algorithm did not help to eleviate completely this stochastic aspect of the model training. As a reasonable solution, it was decided to train the model five times on every train set (cross-validation fold) and save all five models in order to average their predictions on the validation and test sets.

	accuracy	attempt	auc	f1	precision	recall	split
5	0.987063	avg	0.998809	0.984864	0.982880	0.986855	0
11	0.991807	avg	0.999793	0.983550	0.972603	0.994746	1
17	0.978485	avg	0.999591	0.963450	0.998485	0.930791	2
23	0.981043	avg	0.998719	0.968661	0.992701	0.945758	3
29	0.980207	avg	0.995659	0.967514	0.994194	0.942228	4
35	0.977145	avg	0.998376	0.974108	0.992040	0.956814	5
41	0.971959	avg	0.996534	0.958678	0.976684	0.941323	6
47	0.931926	avg	0.974663	0.864957	0.973077	0.778462	7
53	0.942266	avg	0.976872	0.883275	0.969407	0.811200	8
59	0.987500	avg	0.999755	0.979959	0.960705	1.000000	9

Figure 15: Group 10-fold cross-validation metrics

	mean	std
accuracy	0.972940	0.019884
f1	0.952902	0.042598
precision	0.981278	0.012647
recall	0.928818	0.075006

Figure 16: Cross-validation mean metrics

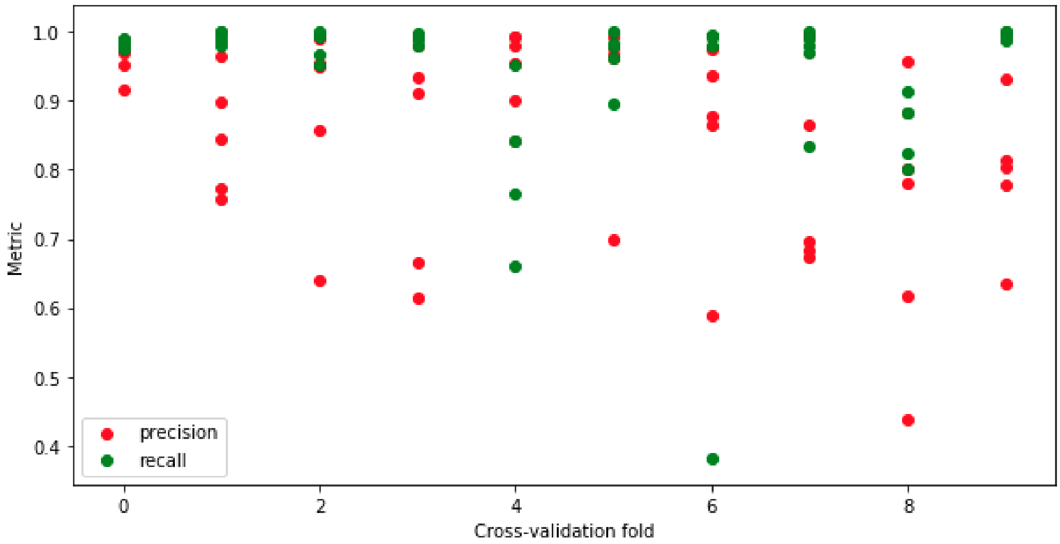


Figure 17: Cross-validation metrics scatter plot

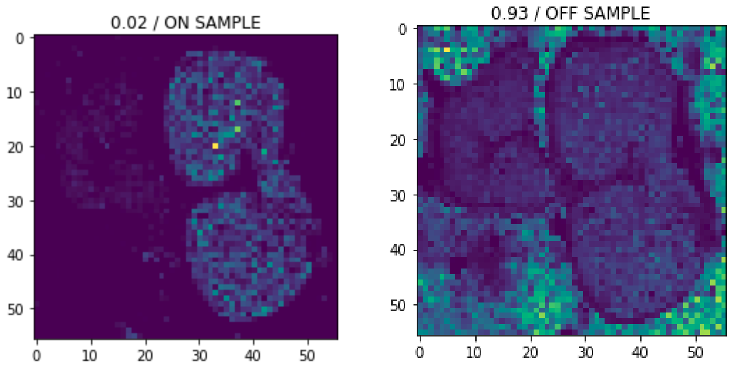
Justification

With use of Deep Convolutional Neural Networks it was possible to solve the problem in focus with higher accuracy ($recall = 0.93 \pm 0.08, precision = 0.98 \pm 0.01$) compared to the benchmark model ($recall = 0.8, precision = 0.75$). As for the original problem precision metric was more important, the final model provided substantially better solution. With achieved accuracy, the final model can be used in METASPACE project for filtering MSI dataset ion images that are not particularly interesting to the user.

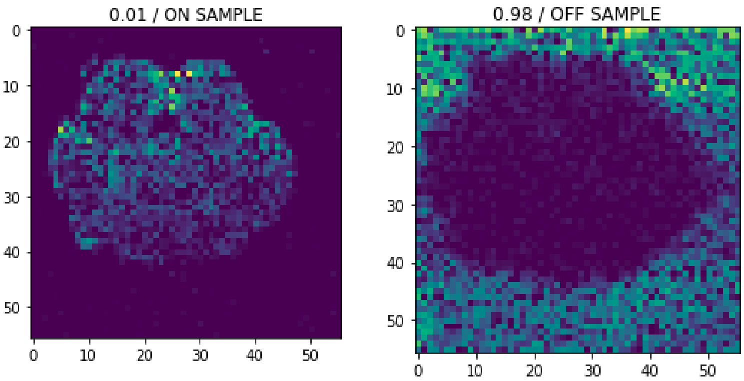
Conclusion

End Result Visualization

Examples of model predictions for a couple of completely new MSI datasets recently uploaded to METASPACE.



(a) MSI dataset 1 predictions with target class probability and assigned label



(b) MSI dataset 2 predictions with target class probability and assigned label

Figure 18: Comparison of on sample ion images (left) with off sample ones (right).

Reflection

The process used for this project can be summarized the following way:

1. Initial problem formulation and setting the project goals.
2. Data exploration and visualisation of separate MSI datasets.
3. Attempts to use unsupervised learning, different versions of PCA and clustering algorithms, for separate MSI datasets in order to group their ion images into two classes.
4. Using K-means clustering of MSI datasets spectra to learn off sample masks for all of them. Using this data as groud truth data to train binary classification models to predict MSI dataset pixel class (on/off sample). Using this model as the benchmark model.

5. Using expert knowledge to collect more than 23 thousands of labeled ion images from more than a hundred MSI datasets.

6. Preprocessing of the ion images: image resizing, experimenting with data augmentation.

7. Coming up with effective schema for the model validation.

8. Optimizing the Convolutional Neural Network architecture and its hyperparameters.

9. Training the final version of the model on all of the labeled data and applying it to some test data (absolutely new MSI dataset from METASPACE).

The most surprising part of the project was to discover that predicting MSI dataset pixel classes with high accuracy is not enough for a good solution of the original problem where classification of ion images is needed. Having a dataset mask of off sample pixels is not enough, one should come up with a smart way of combining this binary mask with each ion image in order to get a single number metric that would say how much the image looks like off sample area. Finding the right threshold for these metrics is also not an easy task as it heavily depends on a MSI dataset.

The most useful part of the project was to try to solve a real world problem from the ground up. From defining the problem itself to comming up with a way to collect input data and labels, to large number of long running experiments with aim to find a network architecture that would work the best. As expected, it was quite difficult to find the right model and its parameter optimal values. But it was suprisingly easy to get from model that kind of works to a completly useless one. So the process of network optimization was definitely the toughest part of the project.

Improvement

Probably the first issue to be addressed for the described solution is the model robustness. The fact that model fits its parameters quite differently on the same train set every time training is done simply does not appeal, especially when one considers moving this solution into a production system. A potential solution can be in finding a better way of initializing network parameters or choosing a better optimizer. Perhaps, exploring other deep learning frameworks can provide other options that are not available in TensorFlow.

One of the issues of the CNN based solution is sensitivity. Model accuracy heavily depends on test MSI dataset. On some of them the result is close to 100%, on others it can be as bad as 50-60%. Using an ensemble of models, with CNN as one of them, will help to exploit different information hidden in the available data. Combining predictions of several independent models might improve the accuracy and robustness of the final solution.