Collagen Fiber Extraction and Analysis in the H&E Dyed Cancer Tissue Images

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Abstract

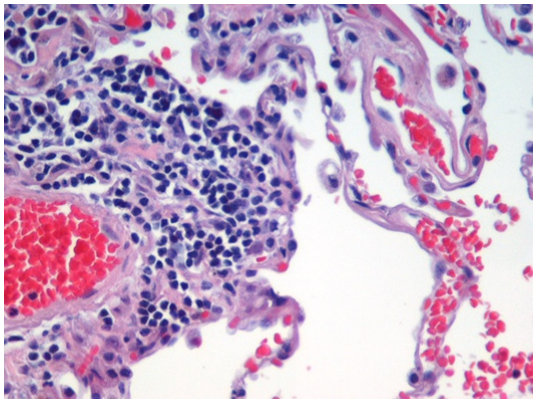
This paper investigates the use of artificial intelligence for predicting cancer patients diagnosis from collagen fibers located in the digital Hematoxylin and Eosin stained tissue images. The proposed technique uses a convolutional neural network to extract a collagen mask from the image. The collagen mask is then fed into a second pipeline that predicts the diagnosis. We conclude that this method is not sufficient to generate a meaningful prediction.

keywords: Convolutional Neural Network, Collagen Fibers.

1 Introduction

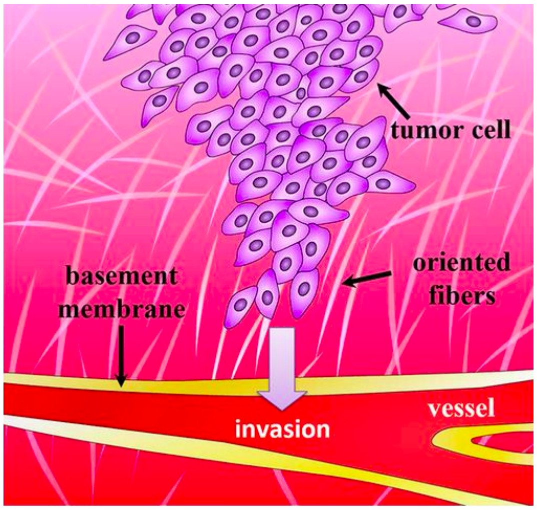
Visual examination of a tissue is a major part in cancer diagnosis. A trained pathologist is given a sample of a tissue to inspect. By looking at the tissue placed under a microscope or inspecting its digital image a pathologist diagnoses the severity of cancer and makes a prediction about the further treatment. Unfortunatelly, manual inspection is prone to error and inter-observer variability (e.g. different pathologists are trained differently). This creates an interest to investigate whether a computer can perform the tissue inspection and provide predictions.

Different components of the tissue must be coloured with different colours so that the visual image of a tissue can be analysed. Hematoxylin and eosin stain (abbreviated as H&E [1]) is one of the principal tissue stains used in histology:



The hematoxylin stains cell nuclei blue whereas eosin stains the extracellular matrix pink, with other structures taking on different shades, hues and combinations of these colors. The stain shows the general layout and distribution of cells and provides a general overview of a tissue sample's structure. It is the most widely used stain in medical diagnosis. When a pathologist looks at a biopsy of a suspected cancer, the histological section is likely to be stained with H&E.

Collagen is one particular component of a tissue. It is a protein located in the extracellular matrix, which becomes pink after the H&E staining. It is believed that the metastatic tumor cells interact with oriented collagen fibers to invade the blood vessels. Several studies have shown a link between collagen remodeling and the invasion and progression of mammary cancer in mouse models [2,3,4]. Furthermore, there was a link observed between collagen morphology, particularly collagen alignment, and breast cancer patient outcome [5]. Provenzano et al.[2] first introduced the so called tumor associated collagen signature (TACS) nomenclature to describe collagen alignment patterns. The TACS phenotypes are currently classified into three groups. TACS-1 describes the standard desmoplastic response of increased collagen deposition surrounding initiating tumor cells. TACS-2 is observed as straightened fibers aligned tangentially around developing tumors. The image is considered to be TAC-3 positive if it contains many straight collagen fibers aligned normally to the epithelial cells boundary regions: [6]



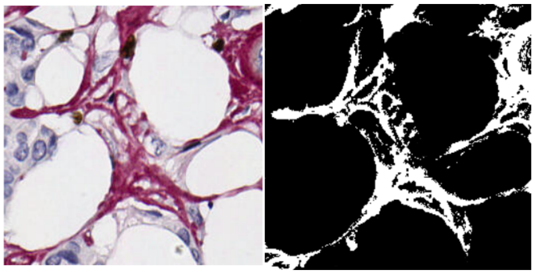
Thus, collagen fibers organisation in the tissue could be used as a biomarker to diagnose the patients cancer severity.

Previous collagen alignment studies were either carried manually, or automated on other imaging techniques, such as the second harmonic generation (SHG) microscopy [7]. However, until the start of this project there was no study that would implement a fully automated pipeline using solely the H&E stained images. This project aims to automate collagen as a biomarker extraction and assesment procedures on the most popular H&E stain.

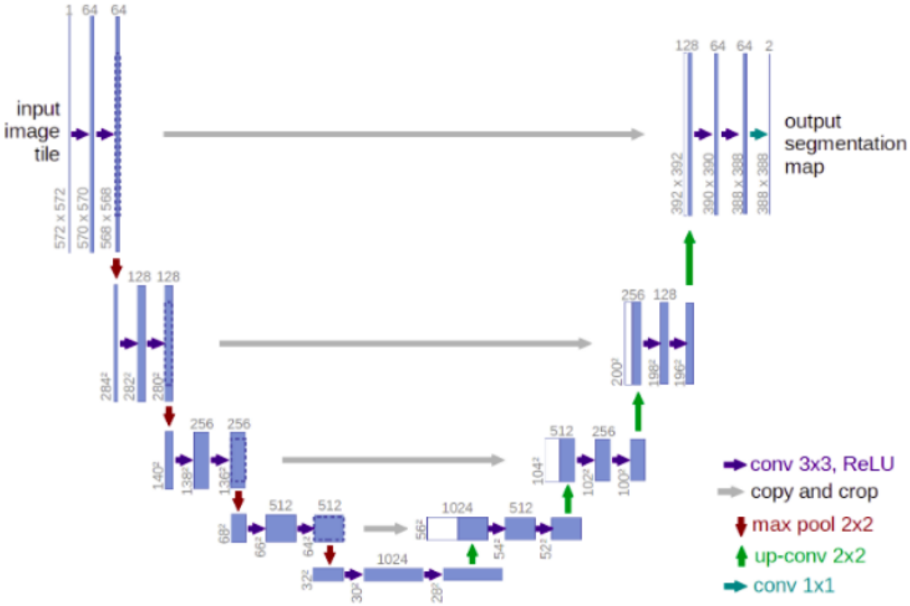
2 Methods

2.1 Collagen Extraction

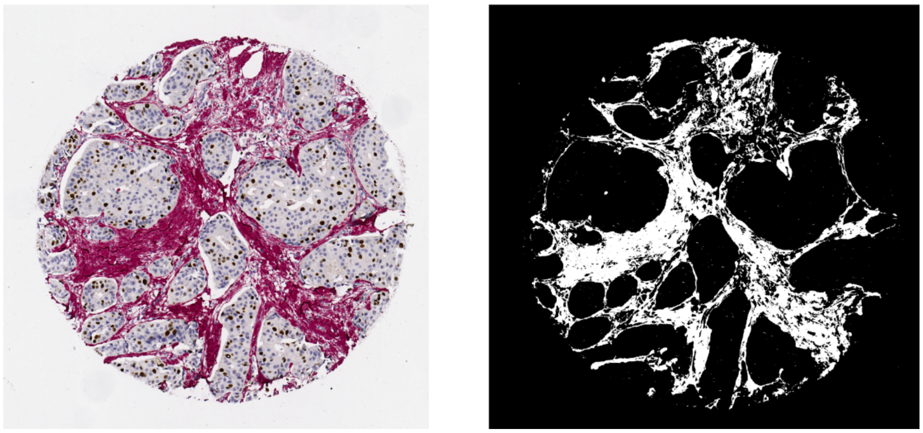
The first step in this project is to take the H&E stained image containing all tissue components and extract only the shape of a collagen present in the image. This is known as the image segmentation problem. In this case a pixel representing the collagen will be labelled as a foreground (bit with value 1), whereas any other pixel will be labelled as a background (bit with value 0). Machine learning was used to solve this problem. A model is created and provided with the training data. The model learns to separate pixels into collagen and background classes and can be applied to unseen images. The training data was provided by the National Center of Pathology, Vilnius. Large H&E images were tiled into tiles of 256×256 pixels and for each tile a collagen mask was manually extracted with a paint software:



In total 43 image-mask pairs were used to train the model. A convolutional neural network U-net was chosen as a model [8]. The model takes a full 256×256 (x3 for each of the RGB colour) image as an input and performs a few downsampling procedures to infer the deep features from the image. Each downsampled layer is then upsampled and connected to the layer above to apply the inferred features on the image. Eventually the model predicts the 256×256 mask. We tried to construct the network with different depth (i.e. the number of downsampling layers). We found that for 256×256 size images a data generated in the 5-th and deeper layers becomes redundant. Thus, a U-net with 4 layers was chosen as the final neural network for the collagen segmentation problem:



The neural network was trained on the 43 image-mask pairs using the cross-validation technique. Binary crossentropy was used as a loss function. Accuracy (i.e. proportion of correct foreground / background pixels) metric was used to test the model. All code was written in python using Keras neural network API [9]. After the model was trained, it was applied on a new larger scale image to test its validity. The larger image was divided into 256×256 tiles and the model was applied to each tile to extract a collagen mask. The extracted masks were then merged into a large mask to obtain the following result:



2.2 Features Generation

After the collagen is extracted, the next step is to analyse it and compute the features that can allow to diagnose the patients. More specifically, a H&E stained image is given corresponding to one patient. We wish to inspect the image and predict the cancer severity stage for that person. The following prediction pipeline is executed for this task:

- A collagen mask is computed from the image. For that the image is split into 256 × 256 tiles and the trained model is applied to each of the tile. The tiles are then merged into a single mask. Each cluster of connected pixels (using 8-connectivity [10]) that has less than 50 pixels is considered a noise and removed from the mask.

- A skeletonization function [11] is applied on the mask. This transforms a white collagen regions into 1 pixel wide representations. The skeleton tree is chopped into individual edges and each edge gets assigned a unique id.

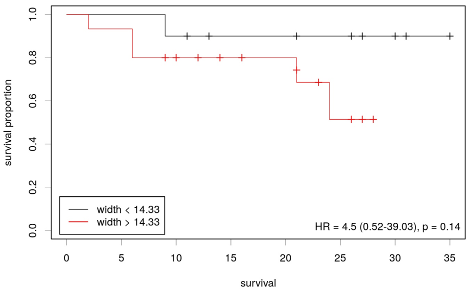
- A breath first search algorithm [12] is then applied to find for each collagen pixel in the mask its closest skeleton edge. In this way the collagen is divided into regions and for each region we can compute its features.

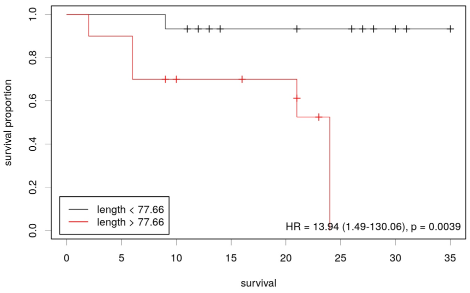
- For each region we compute 16 statistics and average them over all regions. The statistics include region length (computed as the number of pixels in the corresponding skeleton edge), width (computed as the total number of pixels in the region divided by length), collagen to area ratio (computed as the total number of pixels in the region divided by the area of the smallest rectangle enlosing the region) and 13 haralick parameters (excluding the 14th due to its instability) [13,14]. We then use these parameters as features to predict the severity stage of cancer for each patient.

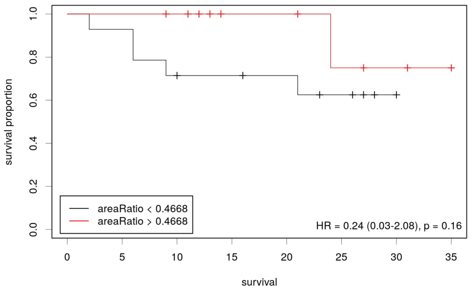
2.3 Diagnosis prediction & Results

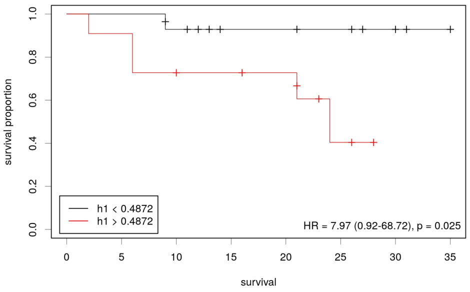
The section above gives multiple features (i.e. a list of numerical values) for each H&E stained image. The final step is to use this feature list to predict the outcome. In this study we used two cohorts for outcome prediction, each cohort acquired from the National Center of Pathology, Vilnius.

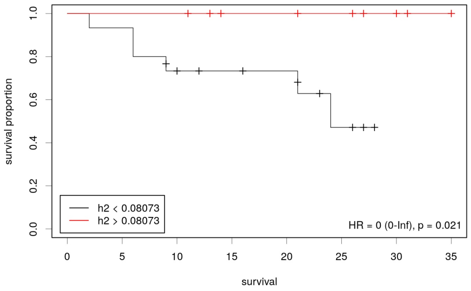
The first cohort comprises 25 large H&E stained images (e.g. 57767×45287 pixels), each image per patient. 6 of them are related with the positive outcome, 19 with the negative. We first compute the 16 statistics mentioned above for each of the patients. To obtain results for this dataset, we perform the Kaplan-Meier analysis [15] on the cohort. For each of the 16 computed features (i.e. a vector of 25 numbers, one vector per feature) we use a Cutoff finder package [16, 17] to derive a threshold for that feature. We then plot two Kaplan-Meier curves based on the threshold: one for numbers less than the threshold, the other for numbers higher than the threshold. We obtain 16 plots in this way:

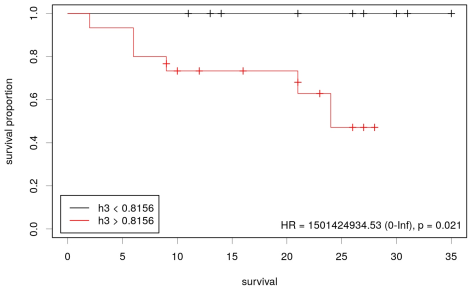


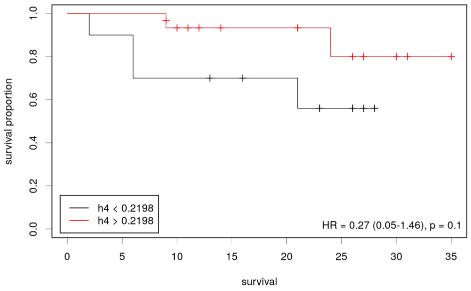


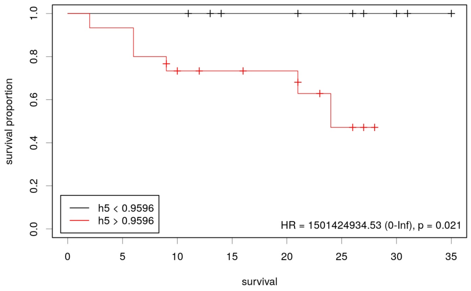


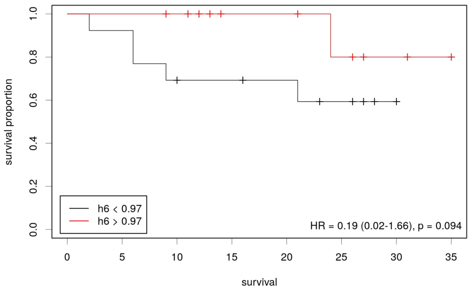


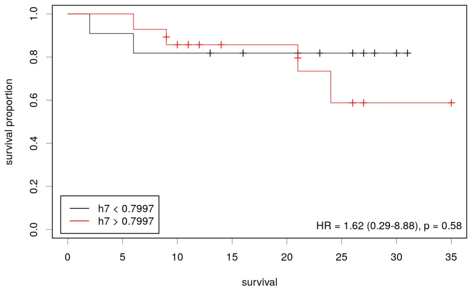

1.0
0.8
0.6
0.4
0.2
0.0
survival proportion
0
5
10
15
20
25
30
35
survival
h2 < 0.08073
h2 > 0.08073
HR = 0 (0-Inf), p = 0.021

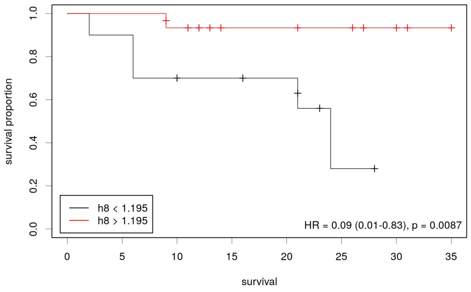

1.0
0.8
0.6
0.4
0.2
0.0
survival proportion
0
5
10
15
20
25
30
35
survival
h3 < 0.8156
h3 > 0.8156
HR = 1501424934.53 (0-Inf), p = 0.021

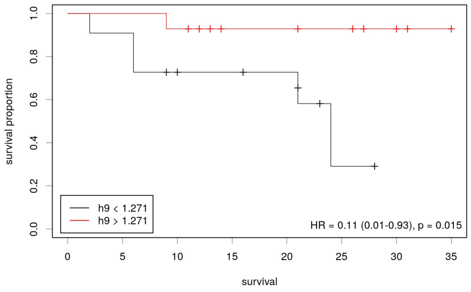

1.0
0.8
0.6
0.4
0.2
0.0
survival proportion
0
5
10
15
20
25
30
35
survival
h4 < 0.2198
h4 > 0.2198
HR = 0.27 (0.05-1.46), p = 0.1

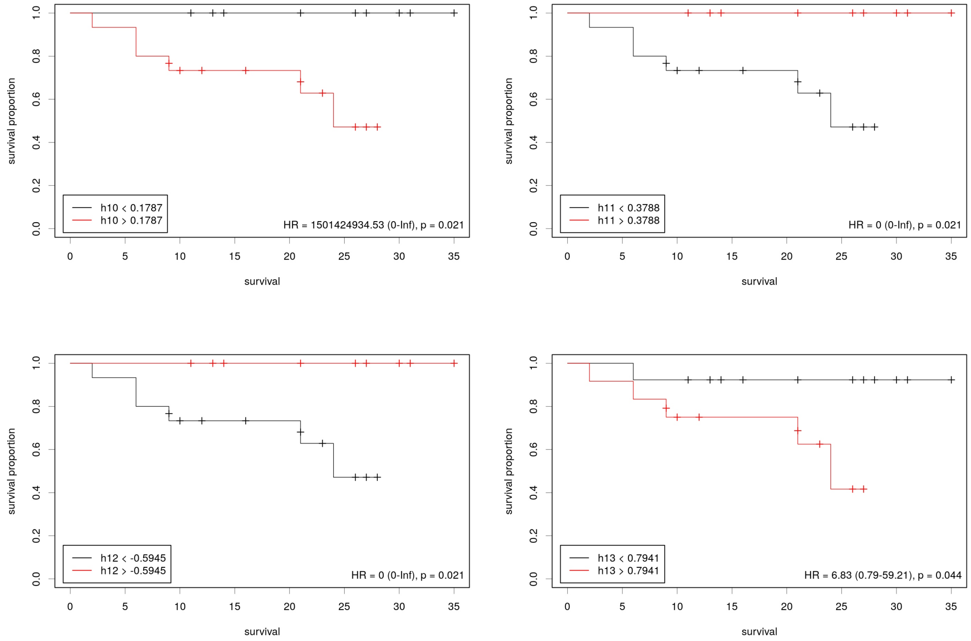

1.0
0.8
0.6
0.4
0.2
0.0
survival proportion
0
5
10
15
20
25
30
35
survival
h5 < 0.9596
h5 > 0.9596
HR = 1501424934.53 (0-Inf), p = 0.021


1.0
0.8
0.6
0.4
0.2
0.0
survival proportion
0
5
10
15
20
25
30
35
survival
h6 < 0.97
h6 > 0.97
HR = 0.19 (0.02-1.66), p = 0.094


1.0
0.8
0.6
0.4
0.2
0.0
survival proportion
0
5
10
15
20
25
30
35
survival
h7 < 0.7997
h7 > 0.7997
HR = 1.62 (0.29-8.88), p = 0.58


1.0
0.8
0.6
0.4
0.2
0.0
survival proportion
0
5
10
15
20
25
30
35
survival
h8 < 1.195
h8 > 1.195
HR = 0.09 (0.01-0.83), p = 0.0087


1.0
0.8
0.6
0.4
0.2
0.0
survival proportion
0
5
10
15
20
25
30
35
survival
h9 < 1.271
h9 > 1.271
HR = 0.11 (0.01-0.93), p = 0.015



11 of these plots (length, h1, h2, h3, h5, h8, h9, h10, h11, h12, h13) report significant p-value scores (less than 5%).

The second cohort comprises 92 TMA spots [18] of size 1500 × 1500, each spot per patient. 71 of them are related with the positive outcome, 21 with the negative. Firstly, we apply the same Kaplan-Meier analysis on the second cohort. No significant features are found in this set.

However, the larger size of this cohort allows us to apply a more sophisticated statistical technique to generate predictions. We augment the data by taking 21 negative images and rotating them by 90, 180 and 270 degrees. This increases the size of the dataset up to $84 + 71 = 155$ samples. We compute 16 features for each sample as before. We then split the data into training and test sets. We train a binary classification model on the training data (a neural network with 2 dense hidden layers of 128 nodes each was chosen) and evaluate it on the test data. We report 51% accuracy on the test set.

The relatively small size of images (1500 × 1500) allows us to try one more approach. We build a second convolutional neural network that takes the whole image as an input. The convolutional neural network has multiple convolution + max pooling layers followed by a pair of dense layers that return a binary output. We again train and evaluate the CNN on a train/test split. We report 54% accuracy on the test set.

3 Conclusions

The study results showed that the visual pattern of the H&E stained collagen in the tissue is too complex for a simple neural network to infer a useful signal for generating a diagnosis prediction. This suggests that to automate the task of predicting a diagnosis from an image, an add-hoc patterns recognised by expert pathologists need to be incorporated into the prediction pipeline.

4 References

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