

Deep Learning Approach for Band Localization in Gel Electrophoresis Images of DNA Extracted from Blood Samples of Hepatocellular Carcinoma Patients

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ABSTRACT

Objective: DNA gel electrophoresis is a molecular biology technique that used for separating different DNA fragments based on their molecular weight. DNA fingerprinting (genetic diagnosis), DNA size estimate and DNA separation for Southern blotting are all applications of DNA gel electrophoresis. Deep learning algorithms can be used to extract both qualitative and quantitative information from images, saving time and effort in interpreting large amounts of data. In addition, computerized data processing and interpretation ensure accuracy and speed without the risk of human error. The aim of this work is to identify DNA bands in each lane of electrophoresis images without the need for any corrections or enhancements to the original electrophoresis image. **Method:** To give a localization to each band within each lane in varied gel images, the proposed model uses mask RCNN with ResNet-50. The accuracy of the suggested model was tested using 16 electrophoresis images with a total of 291 lanes obtained from DNA extraction of hepatocellular carcinoma patients blood.

Results: The proposed model that uses Mask RCNN with ResNet-50 detected all bands within each lane of the gel electrophoresis. The average localization accuracy was 76.6 % and was highly related to the quality of the tested images.

Conclusion: The Mask RCNN with the ResNet-50 model provides a computer-based automated method for band localization without any need for enhancement of the input images.

Keywords: Gel electrophoresis, Deep Learning, mask-RCNN, Band localization, ResNet-50.

1 Introduction

DNA gel electrophoresis (GE) technology is a method to separate DNA fragments, to migrate through a specified substrate, such as a polyacrylamide gel, under the influence of an electric current (Ye et al., 1999). This technique has a wide number of applications, including size estimation of DNA molecules, analysis of PCR amplicons or genotyping, and separation of genomic DNA before Southern analysis (Cai et al., 2017). To perform genetic diagnosis, target DNA sequences are amplified by polymerase chain reaction (PCR). The resulting PCR products (amplicons) are loaded into wells located on top of the gel matrix that indicates lanes for DNA molecules to migrate through the gel medium. At the end of electrophoresis, different sizes of DNA molecules appear as bands in each lane (Intarapanich et al., 2015). These bands can be visualized by DNA stains such as ethidium bromide (agarose gel) or silver nitrate (polyacrylamide gel) (Cai et al., 2017). A scanner is commonly used to capture the band images from the gel slab. The colors on the Gel

Electrophoresis Image (GEI) vary with the dye/stain used, but generally the GEI can be converted to an intensity (or greyscale) image without any loss of information (Akbar Akbari et al., 2010). A GEI might contain one or more gels, each with a number of lanes. Each lane has various bands, corresponding to the presence of DNA molecules with a given molecular weight (Caridade et al., 2009). The intensity of a band depends on the mass (amount, quantity) of DNA. Manual interpretation of banding patterns can be very laborious and inaccurate. Performing large-scale DNA fingerprinting or genotyping thus requires an automated workflow for analysis (A Akbari & Albrechtsen, 2004).

To handle the two key processes of GE analysis, lane and band detection, a variety of imaging processing approaches have been developed. Technical variance due to gel electrophoresis frequently compromises the precision of these procedures (Intarapanich et al., 2015). On distinct DNA gel pictures, a range of image thresholding approaches have been proposed employing a number of common thresholding methods, both global and local (A Akbari & Albrechtsen, 2004). In other studies, multi-thresholding methods are combined with bio-inspired methodologies to offer more accurate lane and band segmentation in gel images (Noor et al., 2011)(Ahmad et al., 2013). All of these thresholding operators produce highly varying outcomes depending on image quality, therefore they can't be applied to all DNA.

Deep learning techniques have increased research interests because of their powerful capability to overcome the drawback of traditional algorithms dependent on hand-designed features (Razzak et al., 2018). Deep learning approaches have also been found to be suitable for big data analysis with successful applications to computer vision, pattern recognition, speech recognition, natural language processing, and recommendation systems (Jin et al., 2021).

In this work, we interested in the analysis of 1D Gel electrophoresis images. There are many software packages available to import and analyze a wide range of one and/ or two-dimensional gel electrophoresis images, including GelPro from Media Cybernetics Inc (*GelPRO Gel Documentation System*, n.d.); EDAS 120 (Electrophoresis Documentation and Analysis System 120) from Eastman Kodak (Bianca, 2000); UVIDoc from UVITEC Inc (*Ultimate, Stand-Alone Geldoc System for DNA & Protein Gel Imaging: Uvidoc HD6*, n.d.), Phoretix 1D/2D gel analysis package from Phoretix International (*Gel Documentation System Software Phoretix 1D Pro*, n.d.), etc.

The main objective of this work is localizing DNA bands in each lane within electrophoresis images without the need of making any correction or enhancement on the input electrophoresis image. Peak positions in one-dimensional profiles can be transferred into the molecular weight domain using the included standard markers in the corresponding gel image. The resulting one-dimensional profiles and their corresponding computed molecular weights could be saved and managed in a proper database. We utilize the use of Mask RCNN with ResNet-50 to detect bands within each lane in various gel images. This research article is divided into 3 sections. Section II discusses the proposed method of band detection of 1D Gel-images. Section III explore and discuss the result analysis of the proposed method, and ultimately, Section IV is associated with the conclusions from the consequences.

2 The Proposed Approach For Gel Electrophoresis Analysis

One-dimensional gel separation is still a major tool for many applications such as clinical and microbial diagnostics, forensic medicine, tissue typing and food safety (Cai et al., 2017). Since gel images take up a considerable storage space, conversion to intensity profiles is very convenient and absolutely required in most high throughput analyses such as microarrays and second-generation sequencing. The proposed method utilizes the use of mask RCNN (He et al., 2017) with ResNet-50 (He et al., 2016) to provide a localization to each band within each lane in various intensity gel images. Due to the limitation of the dataset images we use a transfer learning approach in the training process. The proposed method can be divided into three main phases as shown in figure 1.

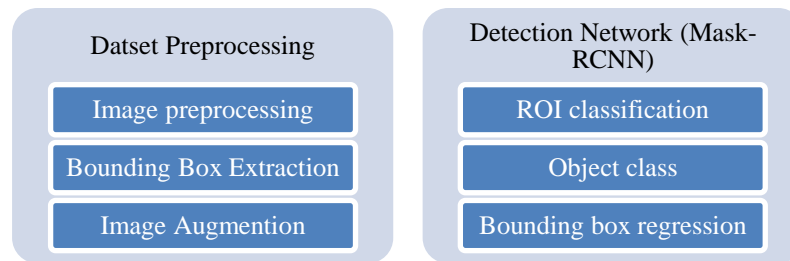


Figure 1: Framework of the proposed Band Localization model

A. Dataset Preprocessing

The experimental detail and methodology of DNA preparation, extraction and gel electrophoresis processing can be found in the published article (Moemen et al., 2019)(Moemen et al., 2018) The VDR gene was amplified by polymerase chain reaction (PCR) techniques followed by restriction fragment length polymorphism (RFLP) assays using allele-specific restriction enzymes *BsmI*, *ApaI*, *FokI* and *TaqI*. The genotype and allelic type were determined by analyzing the banding pattern of the PCR RFLPs images. A GEI might contain one or more gels, each with a number of lanes as presented in (Fig.2) corresponding to the presence of DNA molecules with a given molecular weight. The intensity of a band depends on the mass (e.g. amount, quantity) of DNA. The calculation of the molecular weights and mass for an observed substance is done using a reference in one of the lanes. The reference is a standard substance, with the molecular profile of the various bands known. Before introducing the set of lanes images to mask-RCNN, some preprocessing on the input image and dataset as a whole is required.

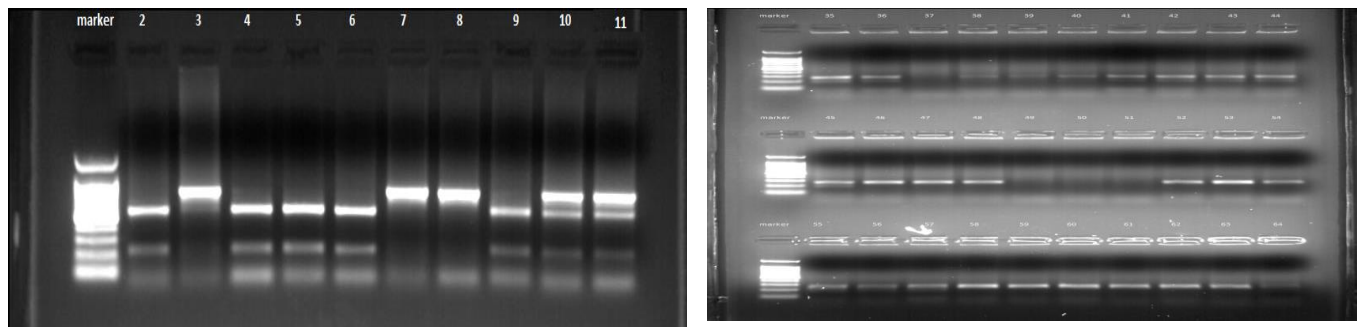


Figure 2: A Samples of GEL image with different lans

Image Preprocessing: Some pre-processing steps are applied to the image before it is sent to the network. The preprocessing phase starts by Subtraction of mean. The mean vector 3×1 , corresponding to 0 each color channel is the mean of pixel values across all the training and test images are subtracted from the input image. After mean subtraction, we propose rescaling Preprocessing Two parameters are taken (target size and maximum size). The shorter side is resized to target size and the longer side is resized accordingly keeping the aspect ratio conserved. However, if the new value for the longer side exceeds the the maximum size, then the longer side is resized to maximum value and resized value of the shorter side is changed with reference to longer side, keeping the aspect ratio conserved. The default values for target size and maximum size are 800 and 1333 respectively. Finally, It is necessary when feature pyramid networks (FPNs) are involved (explained in the next section).

Bounding Box Extraction: In this phase, we extract the bounding box of each object in every image and store the result with four attributes Gel_Id as the name of file and Target as [0 or 1] and (x, y) starting point of the box and (w, h) represent its width and height.

Data Augmentation: We perform three methods of data augmentation (e.g. scaling, Translation, and rotation). Also, we used Gaussian Blur, Contrast Normalization, Additive Gaussian Noise, shear, and Image Sharpe

B. Lane Detection Network

In this work, we proposed the use of mask RCNN as a detection network of Gel bands in each lane. The overall architecture of mask RCNN for band localization within each lane is described in figure 3.

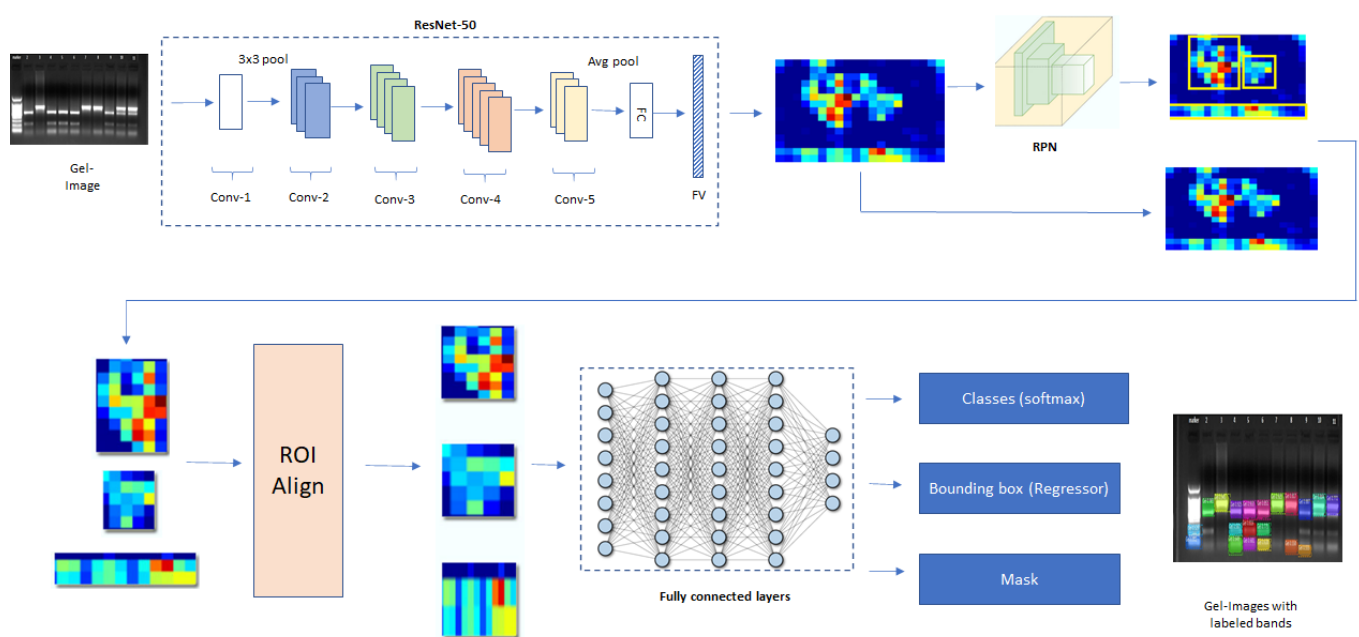


Figure 3: Overall proposed detection RCNN

Mask RCNN: (regional convolutional neural network) is a two-stage framework (He et al., 2017): the first stage scans the image and generates proposals (areas likely to contain an object), and the second stage classifies those proposals and generates bounding boxes and masks. Mask RCNN generates proposals about the regions where there might be an object based on the input image. Mask R-CNN consists of these modules:

Backbone: This is a standard convolutional neural network (typically, ResNet50 or ResNet101) that serves as a feature extractor. The early layers detect low-level features (edges and corners), and later layers successively detect higher-level features (car, person, sky). Passing through the backbone network, the image is converted from 1024x1024px x 3 (RGB) to a feature map of shape 32x32x2048. This feature map becomes the input for the following stages.

Feature Pyramid Network: While the backbone described above works great, it can be improved upon. The Feature Pyramid Network (FPN) was introduced by the same authors of Mask R-CNN as an extension that can better objects at multiple scales. FPN improves the standard feature extraction pyramid by adding a second pyramid that takes the high-level features from the first pyramid and passes them down to lower layers. By doing so, it allows features at every level to have access to both, lower and higher level features. Our implementation of Mask RCNN uses a ResNet50 + FPN backbone.

Region Proposal Network (RPN): The RPN is a lightweight neural network that scans the image in a sliding window fashion, and finds areas that contain objects. The regions that the RPN scans over are called anchors, which are boxes distributed over the image area. This is a simplified view, though, in practice, there are about 200K anchors of different sizes and aspect ratios, and they overlap to cover as much of the image as possible.

First stage: Proposed ROI generation: RPN scans all FPN top-bottom pathway (feature map) and proposes regions that may contain objects. Ground-truth classes (only object or background binary classified at this stage) and bounding boxes are assigned to individual anchors according to some IoU value. As anchors with different scales bind to different levels of the feature map, RPN uses these anchors to figure out where of the feature map should get an object and what size of its bounding box is. Here we may agree that convolving, down-sampling and up-sampling would keep features staying the same relative locations as the objects in the original image, and would not mess them around.

Second stage: ROI Classifier and Bounding Box Regressor

This stage runs on the regions of interest (ROIs) proposed by the RPN. And just like the RPN, it generates two outputs for each ROI. The first output is the class of the object in the ROI. Unlike the RPN, which has two classes (FG/BG), this network is deeper and has the capacity to classify regions into specific classes (person, car, chair, etc.). It can also generate a background class, which causes the ROI to be discarded. The second output is a bounding Box Refinement which is very similar to how it's done in the RPN, and its purpose is to further refine the location and size of the bounding box to encapsulate the object. ROI Pooling ROI pooling refers to cropping a part of a feature map and resizing it to a fixed size. It is similar in principle to a cropping part of an image and then resizing it (but there are differences in implementation details).

Detection Network Configuration: In this experiment, we used a virtual machine with 32 gigabytes of ram, ~~and~~ one Intel Xeon CPU, and NVIDIA Tesla K80 GPU. We start by excluding the last layers because they require a matching number of classes. Second: we train heads with a higher Learning rate = 0.006 to speed up the learning, in this step, there is no need to use any augmented data. We use transfer learning principles (Tang et al., 2020) that instead of training a model from scratch, we start with a weights file that has been trained on the COCO dataset (Tang et al., 2020). the COCO dataset does not contain any Gel classes, but it contains a lot of other images (~120K), so the trained weights have already learned a lot of the low-level features common in natural images.

3 Experimental Results

To evaluate the performance of the presented model, we examined how well the proposed model can localize bands within each lane. Table-1 provides a complete analysis results for all tested images. The dimension of each tested images is reported followed by the total number of lanes, total number of bands in each image, respectively. The (correctly band) localized bands are estimated in the correctly column, followed by the missed bands and the suggested bands. Missed bands refers to bands that was not detected by the proposed model while the suggested bands refers to bands that were detected by mistake as an existing band. The overall accuracy, omission, and commission errors of localization results with each image are listed in the last three columns respectively. The average of these three measures is shown in the last row of table 1. The average localization accuracy for all bands is 76.62 %. This accuracy is highly related to the quality of the tested images. Tested images ~~are~~ vary in background, contrast, and illumination conditions. Figure-4 provides a visual representation of the proposed model for the localized bands for the four electrophoresis images (*BsmI*, *ApaI*, *FokI*, and *TaqI*). The first column shows the original input image, while the second column shows the output of the proposed model with highlighted regions on each detection band.

Table 1: Band localization results

Test Image	Dimension in pixels	No. of lanes	No. of bands	No. of detected bands			Accuracy in %	Omission error%	Commission error %
				correctly	Missed	Suggested			
Apa									
Apal-1	933 x 760	10	29	23	6	0	79	20	0
Apal-2	933 x 758	26	47	33	14	4	70	29	10
Apal-3	939 x 816	34	38	33	5	0	86	13	0
Bsm									
Bsml-1	933 x 552	17	39	30	9	7	76	25	18
Bsml-2	933 x 394	17	28	18	10	3	64	35	14
Bsml-3	933 x 774	20	24	19	5	3	79	20	13
Bsml-4	938 x 448	10	11	9	2	1	81	18	1
Fok									
Fok-1	939 x 748	17	27	18	9	3	66	33	14
Fok-2	933 x 670	10	12	9	3	4	75	23	33
Fok-3	939 x 784	17	17	15	2	10	88	11	40
Fok-4	836 x 901	39	33	33	0	24	100	0	42
Taq									
Taq1-1	939 x 748	17	24	13	11	0	54	45	0
Taq1-2	938 x 406	7	13	10	3	4	76	23	28

Taql - 3	939 x 748	17	49	26	23	7	53	46	21
Taql- 4	939 x 784	30	39	31	8	2	79	20	6
Taql - 1	933 x 730	9	9	9	0	7	100	0	43
Average							76.62 %	22.53 %	17.68 %

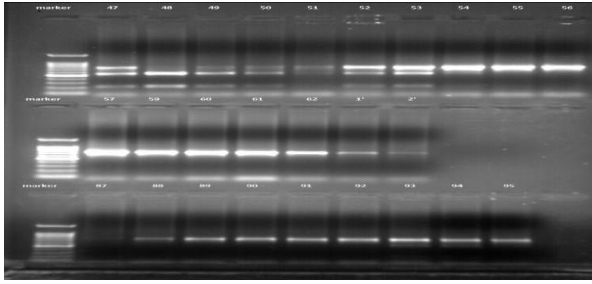


Figure 4: Samples of localization lanes by the proposed model

4 Conclusions and Future Work

When a significant number of bands are manually interrogated, accurate interpretation of DNA banding patterns from electrophoretic images can be time-consuming and error-prone. This work introduced a novel model of band localization in electrophoresis images using a deep learning network. The proposed model uses Mask RCNN with ResNet-50 to detect bands within each lane in various gel images. The proposed model provides a completely automatic method for band localization without any enhancement on the input images. The model use transfer learning to provide high accuracy results despite a small dataset. This accuracy can be improved by providing some image enhancement techniques on the input images before the learning phase to eliminate any variations. In the future, the resulting bands can be subject to some qualitative and quantitative analysis as a second step of automatic detection.

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