# CELL ABUNDANCE AWARE DEEP LEARNING FOR CELL DETECTION ON HIGHLY IMBALANCED PATHOLOGICAL DATA

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# **ABSTRACT**

Automated analysis of tissue sections allows a better understanding of disease biology, and may reveal biomarkers that could guide prognosis or treatment selection. In digital pathology, less abundant cell types can be of biological significance, but their scarcity can result in biased and sub-optimal cell detection model. To minimize the effect of cell imbalance on cell detection, we proposed a deep learning pipeline that considers the abundance of cell types during model training. Cell weight images were generated, which assign larger weights to less abundant cells and used the weights to regularize Dice overlap loss function. The model was trained and evaluated on myeloma bone marrow trephine samples. Our model obtained cell detection F1-score of 0.78, a 2\% increase compared to baseline models, and it outperformed baseline models at detecting rare cell types. We found that scaling deep learning loss function by the abundance of cells improves cell detection performance. Our results demonstrate the importance of incorporating domain knowledge on deep learning methods for pathological data with class imbalance.

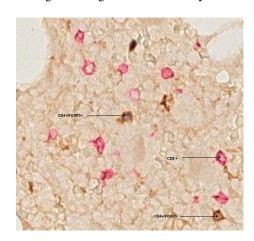
**Index Terms**— Deep learning, convolutional neural network, cell detection, class imbalance, digital pathology, multiplex immunohistochemistry.

# 1. INTRODUCTION

In digital pathology, cell detection and classification are the first step to assessing tumour load, surrounding microenvironment and immune phenotypes [1]. Multiplex immunohistochemistry (mIHC) is a staining method that allows simultaneous examination of multiple cell markers in a single image, where each cell is represented by a unique color or color combinations (Fig. 1). Intrinsically, some cell types are fewer compared to others. For example, in bone marrow trephine samples, the number of CD4+/FOXP3- effector and CD4+/FOXP3+ regulatory T cells is lower than that of CD8+T cells (Fig. 1). This imbalance causes instability and bias

on the performance of discriminative models.

Recently, different deep learning techniques have been proposed to address the issue of class imbalance in medical image data. Some methods focus on sampling, and/or augmentation [2]. The sampling method reduces variability of the data [2]. Both methods are suited for segmentation (for example, background vs. foreground segmentation) and classification applications because a training sample for such applications has fewer number of instances/labels. However, in a patch based cell detection, there might be hundreds of cells in a small patch belonging to different classes. Thus, patch level sampling and augmentation approaches might increase the degree of imbalance in the context of single cell detection. Other methods focused on developing a robust training loss function [2, 3]. Folk et al. [3] proposed an approach that assigns cell weight from cell segmentation. However, collecting manual single cell segmentation is costly.



**Fig. 1**. Samples mIHC image showing class imbalance. The number of CD8+ cells (red) are higher than CD4+/FOXP3-(brown) and CD4+/FOXP3+ (dark blue) cells.

In this work, we proposed a new class balancing approach in the context of single cell detection from single cell dot an-

**Table 1.** Distribution of dataset. number of slides  $(N_s)$ 

_	$N_s$	CD8+	CD4+/FOXP3-	CD4+/FOXP3+
Training	5	2244	997	243
Validation	3	1555	689	140
Test	3	1306	702	138

notation. Our work has the following contributions:

- We implemented cell detection and classification deep learning framework that uses class balancing technique of Dice overlap in dataset with class imbalance.
- We implemented an algorithm that generates cells weight image from expert dot annotation based on the relative abundance of cell types in the training data.
- We implemented and compared performance of different cell weighting strategies.

#### 2. MATERIAL

Our dataset contains 11 newly diagnosed myeloma bone marrow mIHC whole slide images. It contains CD8+, CD4+/FOXP3- and CD4+/FOXP3+ cell types (Fig. 2a). To train and evaluate the proposed method, a total of 8014 cells were annotated in different regions of the whole slide images by experts by putting a dot at the center of a cell (Fig. 2b). Table 2 shows training, validation, and testing split.

# 3. METHODOLOGY

# 3.1. Cell detection training data preparation

For training, the annotated regions were divided into 256x256 x3 patches. Let n be the number of training patches, the training data,  $T_d$  is represented by a set  $T_d = \{\mathbf{I}, \ \mathbf{R}, \ \mathbf{W})\} = \{(I^1, R^1, W^1), (I^2, R^2, W^2), (I^3, R^3, W^3), ..., (I^n, R^n, W^n)\}$ , where  $I^i \in R^{256 \times 256 \times 3}, W^i \in R^{256 \times 256 \times 1}$ , and  $R^i \in R^{256 \times 256 \times 1}$  are the  $i^{th}$  input, weight, and reference images, respectively. Sample I, R and W images are shown in Fig. 2b.

**Reference image:** it is an artificial image generated from the expert single cell dot annotation using Equation (1).

$$R(i,j) = \begin{cases} 1 & \text{if } d < r \\ 0 & \text{otherwise} \end{cases} \tag{1}$$

where R(i,j) is pixel value at (i,j) and d is an Euclidean distance from (i,j) to the closest cell center. The value of r was set to 4 pixels (1.768  $\mu$  m). The value of r was chosen empirically, making sure blobs in R don't touch each other (Fig. 2b).

Weight image: it assigns a weight to every cell in the input image (Fig. 2b). The weights are inferred from the relative abundance of each cell type in the training data. Rare cells are given larger weight. Let n be the number of cell types in the training dataset, let  $C = \{c^1, c^2, c^3, ..., c^n\}$  be the n cell types, and let  $N^k$  be the number of  $c^k$  cells in the training data. Then,  $N = \{N^1, N^2, N^3, ..., N^n\}$  represent a set of abundance of cells for the n cell types. Weight image is generated using information from N and location of cells. We implemented three cell abundance weighting functions: RatioWeight (Equation 2), ExpWeightType1 (Equation 3), and ExpWeightType2 (Equation 4).

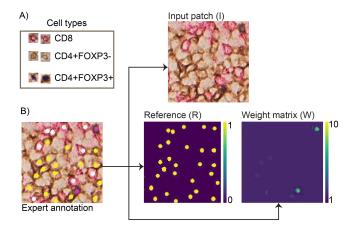
$$W(i,j) = \begin{cases} \frac{max(N)}{N^k} & \text{if } d^k < r\\ 1 & otherwise \end{cases}$$
 (2)

$$W(i,j) = \begin{cases} \exp{-\frac{N^k}{max(N)}} & \text{if } d^k < r\\ \exp{-1} & otherwise \end{cases}$$
 (3)

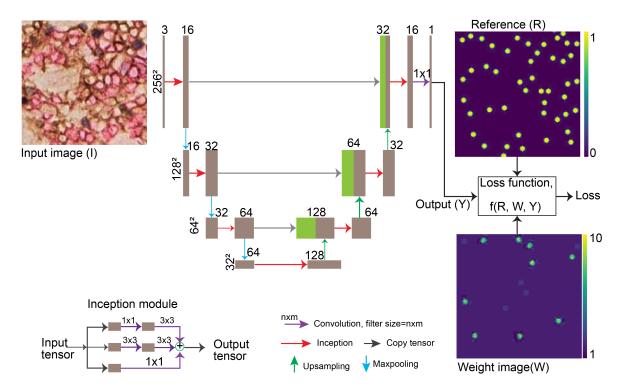
$$W(i,j) = \begin{cases} \exp{-(\frac{N^k}{max(N)})^2} & \text{if } d^k < r\\ \exp{-1} & otherwise \end{cases}$$
 (4)

where  $d^k$  is an Euclidean distance from (i, j) to the  $k^{th}$  cell type center. The value of r was set to 4 pixels (1.768  $\mu$ m).

For our dataset,  $C = \{CD8+, CD4+/FOXP3-, CD4+/FOXP3+\}$ ,  $N = \{2244, 997, 243\}$  and these values are used to generate the weights using Equation (2 - 4).



**Fig. 2**. Training data preparation. a) Sample patches for all cell types. b). Sample annotated, reference (R) and weight (W) images for an input image (I). In W, less abundant cell type is assigned larger weight. CD4+/FOXP3+ cells have larger weight than CD4+/FOXP3- and CD8 cells.



**Fig. 3**. Schematic of cell detection model. The number on the top and side of the blocks indicate the size and spatial dimension of the features, respectively. The loss function is computed using model output, reference and weight images.

#### 3.2. Cell detection model

Our proposed cell detection pipeline is shown in Fig. 3. It is a U-net [4] convolutional neural network (CNN) inspired by inception V3. We applied inception V3 blocks which extracts multi-scale features at a given layer. The model has encoder and decoder part. The encoder learns low dimensional representation of the input image, and the decoder reconstruct a target image. The 1x1 convolutional layer at the end of the architecture transforms 256x256x16 dimensional features to 256x256x1, size of reference image (R). Parameters were initialized using uniform glorot [5], and optimized using Adam [6] with learning rate of  $10^{-4}$ . ReLU activation was applied to all layers, but Sigmoid to the last layer to transform the output to probability.

#### 3.3. Cell detection loss function

To minimize the effect of class imbalance, we applied weighted dice overlap loss. The loss was computed as

$$l = 1 - 2 \frac{\sum_{j=0}^{j=w} \sum_{i=0}^{i=h} W(i,j) Y(i,j) R(i,j) + \epsilon}{\sum_{j=0}^{j=w} \sum_{i=0}^{i=h} W(i,j) (Y(i,j) + R(i,j)) + \epsilon}$$
 (5)

where W, R and Y are the weight, reference and output images, respectively. w and h represent width and height of input image respectively.  $\epsilon=10^{-5}$  was added to ensure computational stability.

#### 3.4. Cell classification model

To train a cell classification model, we extracted 28x28x3 patches as shown in Fig. 2a. We applied VGG [7] style architecture, which contains three convolutional layers with  $\{16, 32, 64\}$  filters followed by two dense layers with  $\{200, 3\}$  neurons. ReLU activation was applied to all layer, but Softmax for the last layer to transform the tensors to probabilities. Parameters were initialized using uniform glorot [5], and optimized using Adam [6] with learning rate of  $10^{-4}$ . We applied categorical cross entropy loss with class weighting explained in Equation (2-4).

# 4. RESULTS AND DISCUSSION

To evaluate the performance of the proposed different weighting strategy based cell detection models and compare with other state of the art U-Net [4] and CONCORDe-Net [8], we measured precision, recall, and f1-score on separately held test images. CONCORDe-Net [8] is cell count regularized CNN designed for cell detection for mIHC images.

F1-score of 0.78 was obtained using ExpWeightType1 and RatioWeight models, a 2% increase compared to U-net and 1% increase compared to CONCORDe-Net [8] as shown in Table 2. Moreover, recall of ExpWeightType2 model was higher than baseline models by at least 5%. For ExpWeightType1 model, a detection was considered as true positive if it

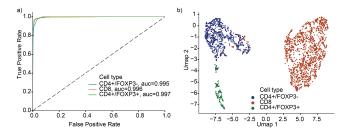
is within 10 pixels  $(4.42\mu m)$  Euclidean distance to a ground truth annotation. For all models, the distance was optimized independently maximizing F1-score. This suggests class weighting improves cell detection performance.

**Table 2.** Cell detection performance of different models. Unnet [4] model is a model in Fig. 3 trained without applying weights.

Method	Precision	Recall	F1-score
ExpWeightType1	0.82	0.75	0.78
RatioWeight	0.80	0.75	0.78
ExpWeightType2	0.78	0.77	0.77
CONCORDe-Net [8]	0.81	0.72	0.76
U-net [4]	0.80	0.70	0.76

To measure classification performance, we measured area under the curve (AUC) and accuracy on a separately held test images. For cell classification, there was no significant difference on AUC for the different weighting strategies. Fig. 4a shows the performance of cell classifier with ExpWeight-Type1 weighting. AUC was greater than 0.995 for all cell types. Overall accuracy of 0.97 was achieved.

To visualize separability of cell types using deep learnt features and to scrutinize miss-classified cell types, we applied uniform manifold approximation and projection (UMAP) dimensionality reduction (Figure 4b). The different cell types are mapped into different UMAP space in 2D. CD8 cells in the same space with CD4 cells are cells expressing both CD4 and CD8 proteins.



**Fig. 4.** Cell classification model performance evaluation. a) Receiver operating characteristics curve and area under the curve (AUC). b) UMAP visualization of 200 dimensional deep learned features.

In our dataset, compared to CD8+ cells there are less number of CD4+/FOXP3+ cells. The visualization in Fig. 5 indicates a model with ExpWeightType1 detected CD4+/FOXP3+ cells, which were under-represented in the training dataset, while the model trained without any weight (U-net) missed some of these cells. For CD8+ cells, the detection results remain similar with and without cell weighting. However, we observed that the weighted model sometimes failed to detect large (in size) CD4+/FOXP3+ cells. This could be because

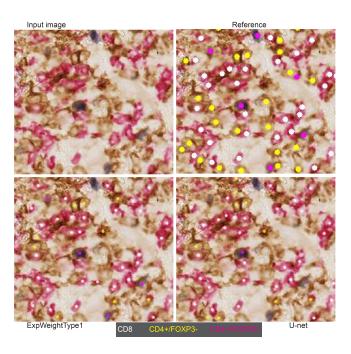


Fig. 5. Samples results from different cell detection methods.

of under-representation such type of cells in the training data. This indicates the proposed weighting method introduces as attention mechanism to the model to detect rare cell types and thus improve overall cell detection performance.

For reproducibility, model was trained using a Docker image within Singularity container on HPC cluster. Code is available at <a href="https://github.com/YemanBrhane/AwareNet">https://github.com/YemanBrhane/AwareNet</a>. The docker image is available on at <a href="https://github.com/YemanBrhane/AwareNet">yhagos/tf2gpu:concordenet</a> on Docker Hub.

Our study has limitations. Our samples were collected from different hospitals with potential differences in processing and fixation, but they were stained and scanned using the same protocols and platform. We trained and validated the model on small scale dataset.

Overall, our results demonstrate the importance of incorporating domain knowledge for deep learning training on a dataset with class imbalance. In the future, we will apply the model on a larger cohort of bone marrow samples, to understand the composition of the bone marrow immune microenvironment, and the changes imposed by malignant disease.

# 5. CONCLUSION

In this paper, to minimize the effect of cell imbalance in cell detection, we proposed a deep learning method that considers abundance of cells during training. Cell weight images were generated by assigning larger weights to less abundant cell types and applied the weights to regularize Dice overlap loss function. Using negative exponential weighting, we obtained a 2% increase in cell detection F1-score, and better rare cells

detection compared to baseline models.

# **Compliance with Ethical Standards**

The data used in this study was obtained with appropriate ethical approval granted by HRA and HCRW (REC Reference 07/Q0502/17).

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