Red Blood Cell Artifacts Identification in Multiplexed Immunofluorescence Image Using Deep Learning

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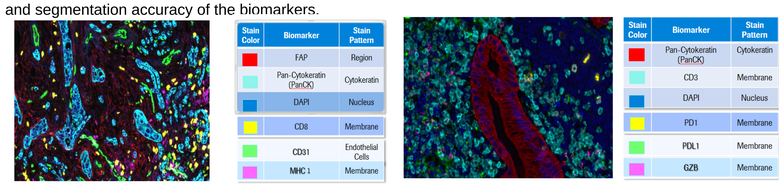
Background

Multiplexed Immunofluorescence (MPX) staining of tissue sections allows simultaneous detection of multiple biomarkers and their co-expression at individual cell level (Fig. 1). However, red blood cell (RBC) artifacts generated by auto fluorescence interfere with image analysis in multiple ways (shown in Fig. 2), which can cause: (1) false detection of nuclei in the DAPI channel; (2) misclassification of individual biomarkers; (3) incorrect segmentation of tumor areas. The objective of this study was to identify RBC artifacts to improve the classification and segmentation accuracy of the biomarkers.



Stain Color	Biomarker	Stain Pattern
	FAP	Region
	Pan-Cytokeratin (PanCK)	Cytokeratin
	DAPI	Nucleus
	CD8	Membrane
	CD31	Endothelial Cells
	MHC 1	Membrane

(a) (b)

Stain Color	Biomarker	Stain Pattern
	Pan-Cytokeratin (PanCK)	Cytokeratin
	CD3	Membrane
	DAPI	Nucleus
	PD1	Membrane
	PDL1	Membrane
	GZB	Membrane

(c) (d)

Figure 1: Examples of multiplex Images in two panels

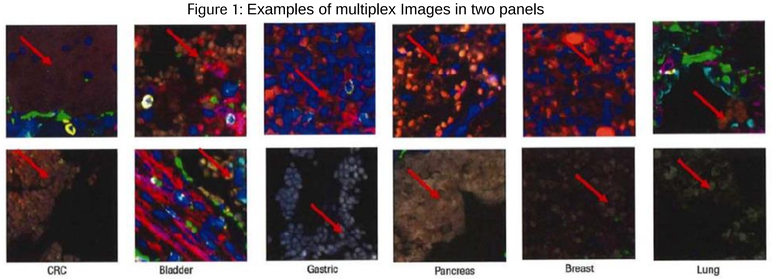
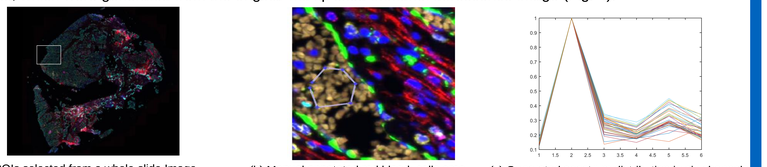


Figure 2: Examples of red blood cell artifacts in multiplexed immunofluorescent images of different types of tissues

Methods

1. Ground Truth Generation: The users selected regions of interest with RBC candidates from a MPX whole slide image (Fig. 3 (a)), then drew annotations for the RBC candidates in 20X/40X Fig. 3 (b). Then, the algorithm analyzed the spectrum analysis in these annotated regions in Fig. 3 (c). Figure 4 describes the procedure of generating ground truth patches. The image patches of size 256X256 were extracted from the field-of-view color images, which were generated from the original multiplexed immunofluorescence image (Fig. 4).



(a) ROIs selected from a whole slide Image (b) Manual annotated red blood cells area (c) Generated spectrum distribution in six channels

Figure 3: Ground truth image selection of MPX images

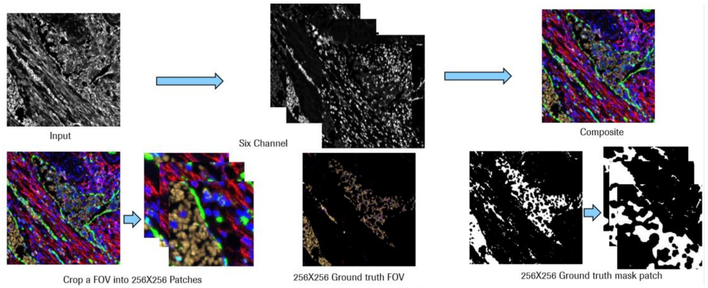


Figure 4: Ground truth patch generation of MPX images

Methods

2. Deep learning Network: The machine learning (ML) execution handler received multi-channel raw data (e.g., channel images) of the MPX image. The linear mapping was performed to generate a composite image of MPX image. The composite image was input to the classifier, which is a U-Net [1] model to identify RBC artifacts. As purely intensity-based methods for RBC identification have limited accuracy, a deep convolution-neural-network based method for image segmentation (U-Net) was explored because it incorporated intensity as well as texture features through learning from image data. Field-of-view images were obtained from the whole slides, on which ground truth region masks were marked for RBC artifacts and non-RBC objects. Figure 5 displays the u-network used in this study. The details are as follows: Binary cross entropy is selected as the loss function. Training epoch is 50 and the learning rate is 1e-5. Batch size is 2 and the optimizer is Adam.

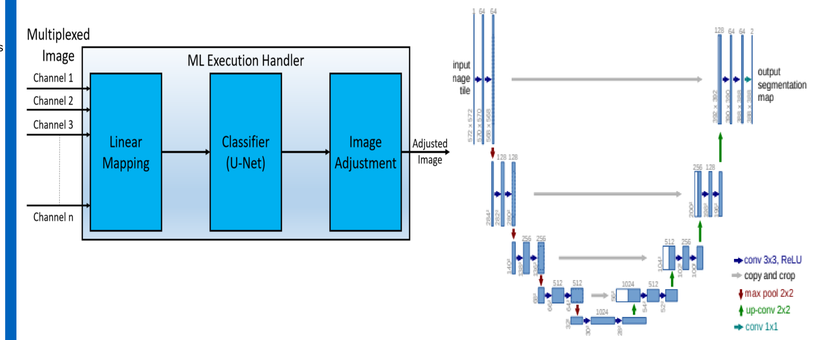


Figure 5: U-Net for red blood cell artifacts segmentation

Methods

3 Three experiments: Three experiments were performed: (1) using a single tissue indication (Bladder, 152 patches) to train and test the network.; (2) using a combination of six tissue indications (Colon, Lung, Breast, Pancreas, Gastric, and Bladder, 275 patches); (3) combining six indications and negative patches where no RBC signal present (344 patches). In all experiments, 80% of the data were used for training and 20% of the data were used in this study. Table 1 describes the testing datasets in three databases.

Indication	CRC	Bladder	Breast	Pancreas	Lung	Gastric	All other Negative
No. of Patches	20	152	10	27	20	46	69

Table 1: The database used in this study

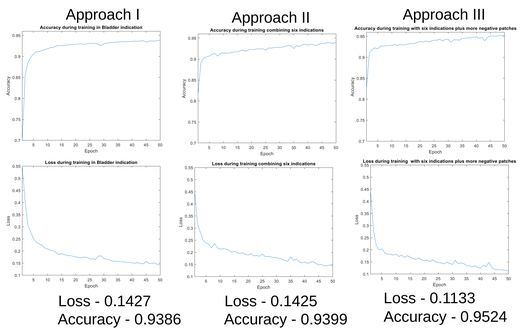


Figure 6: Training performance on three training datasets.

Results

Compared to the annotated ground truth, the training accuracy in the three experiments was 93.8%, 93.9%, and 95.2%, respectively. The training loss was 0.142, 0.142, and 0.113, respectively (Fig. 6). The accuracy for the test data in the three experiments was 85.6%, 85.5%, and 88.3%, respectively. The loss for the test data was 0.149, 0.148, and 0.119, respectively. Four field-of-view images were randomly selected from the test dataset; the pixel-to-pixel agreement between the identified RBC mask and the ground truth mask were 84% to 100% (Fig. 7).

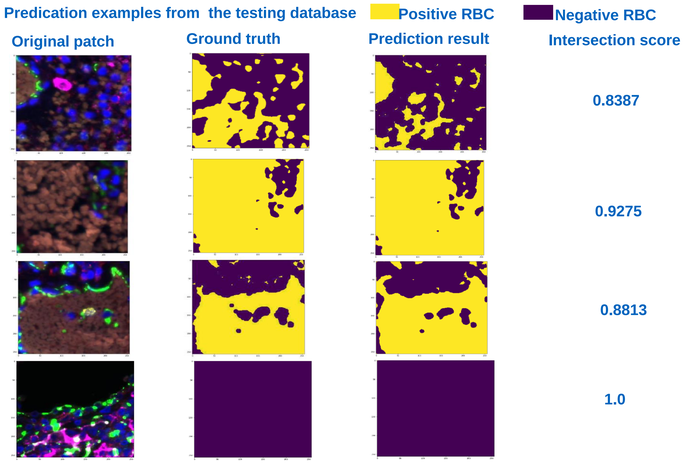


Figure 7: Four predication examples from the testing database

Conclusions

The proposed method has been shown to perform well in identifying RBC artifacts in multiplexed immunofluorescence images. More diversified ground truth data including negative samples improved the overall performance. Meanwhile, this method did improve our ability to detect biomarkers more accurately in MPX slides.

Future work

Training and testing on composite images generated visually reasonable results, future work includes:

- Use larger datasets containing more diversified ground truth
- Compare the performance of deep learning with traditional machine learning approach
- Use six channel (256X256X6) images Instead of composite image (256X256X3) to train model (Fig. 8)
- Extend this method to identify more auto-fluorescent artifacts in addition to RBC artifacts (for example, auto-fluorescing biological elements, such as fat, tissue, or connected tissue).

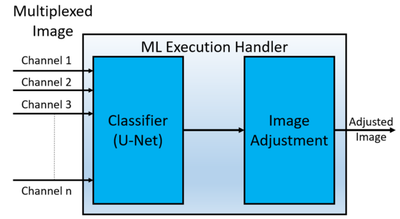


Figure 8: Use six channel raw image to train and test to identify red blood cell artifacts

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References

1.Ronneberger, Olaf, Fischer, Philipp, Brox, Thomas, U-Net: Convolutional Networks for Biomedical Image Segmentation, Computer Vision and Pattern Recognition ,2015.