Classification Beats Regression: Counting of Cells from Greyscale Microscopic Images based on Annotation-free Training Samples

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* **Equal contribution.**

Introduction

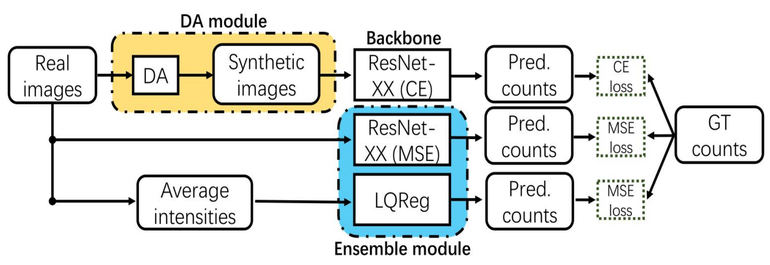
The counting of cells from microscopic images is usually formulated as a regression problem, which often regresses the cell count on a microscopic image or some high-level features extracted from the image.

There are two drawbacks of this formulation:

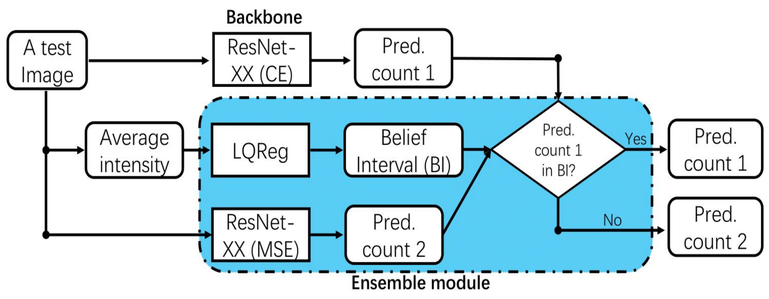
1. They often require manual annotation (e.g., dot annotations indicating the centroids of cells or segmentation masks identifying the contours of cells).
2. The predicted cell counts often deviate from the ground truth even for some simple test images.

To address these drawbacks, we propose a supervised learning framework based on classification-oriented CNNs to count cells without using manual annotations. Our proposed framework achieves the state of the art on the Tiny-BBBC005 dataset and won the Case Study 1 competition of the annual meeting of Statistical Society of Canada.

Workflow



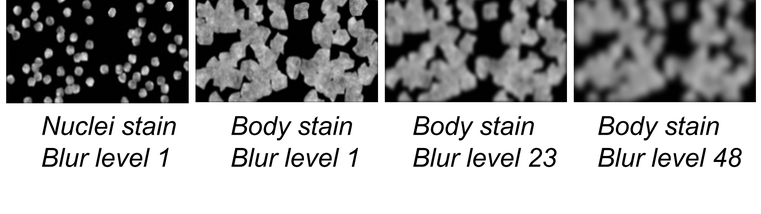
Training stage.



Testing stage.

Dataset: Tiny-BBBC005

- Simulated greyscale microscopic images.
- There are 24 distinct cell counts in [1,100].
- 2 stain types and 3 blur levels.
- For each combination of the blur level, stain type, and cell count, there are 25 images.
- 2400 images in training, 1200 in testing.



Nuclei stain Blur level 1 | *Body stain Blur level 1* | *Body stain Blur level 23* | *Body stain Blur level 48*

Related Work

- DRDCNN[1]: a U-Net is trained to extract the dot density map in I; then regresses the cell count on the density map via a VGG-19.
- FPNCNN[2]: a FPN to generate the segmentation mask for cells in a given microscopic image in I, then regresses the cell count on this mask via a VGG-19.
- ERDCNN[3]: combination of DRDCNN and FPNCNN, the cell count is regressed on an integration of the dot density maps and the segmentation masks.
- Regression-oriented ResNet[4]: directly predict the cell count from a microscopic image by training a ResNet that minimizes the mean squared error (MSE) loss.

Method

We formulate the cell counting from microscopic images as a classification problem, where the cell counts are taken as class labels. We train classification CNNs by minimizing the cross-entropy (CE) loss, and use the trained CNN to predict cell counts. But there may be two limitations with this formulation:

1. Some cell counts in the test set may be missing in the training set, prediction errors on these test samples are inevitable.
2. The ordinal information in the cell counts is not utilized.

Data Augmentation (DA)

To address the first limitation, synthetic images for unseen cell counts are created based on real images. Given distinct images and their cell counts, a synthetic image can be created by overlaying real images. For example, a synthetic image with 15 cells can be created by overlaying two real images with 5 and 10 cells respectively.

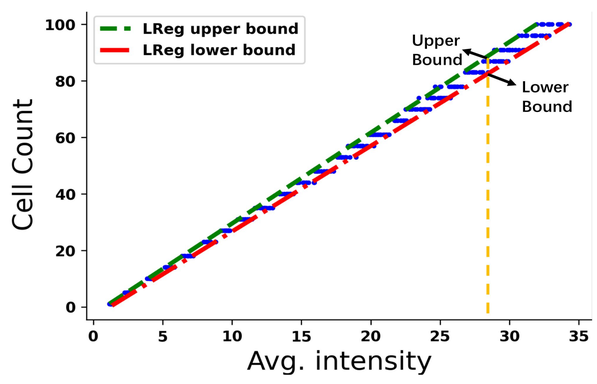
5 Cells (real) + *10 Cells (real)* = *15 Cells (synthetic)*

For a given unseen cell count, we create a pool of potential formulae. One synthetic image with this unseen cell count is synthesized at a time by randomly choosing one formula from the pool. Based on the belief interval introduced below, the synthesized images, whose average intensity fall outside the belief interval, are removed.

Ensemble Classification & Regression

We propose an ensemble scheme that combines the high precision of classification oriented ResNets on seen cell counts with the high stability of regression oriented ResNets on both seen and unseen cell counts.

1. Predict the cell count by ResNet-XX (CE).
2. If the predicted cell count from ResNet-XX (CE) is outside the **belief interval**, we then use ResNet-XX (MSE) to make the prediction.



Scatter plots (blue dots) of the nuclei/body stained and blur level 1 with linear/quadratic regression lines. The green and red regression lines provide a belief interval for the ground truth cell count of any given image.

Benefits:

1. Moderate the influence of unseen cell counts on classification CNN, even when we do not know which counts are missing.
2. The belief interval implicitly utilizes the ordinal information in the cell counts and ensures that the classification CNN does not make "big" mistakes.

Experiment & Results

Exp 1: No Unseen Counts

All images in the training set are used.

Table1. *Test RMSE and MAE in Exp 1. The DA and ensemble module are disabled in this experiment.*

Method	Annot. Type	Pred. Type	RMSE	MAE
DRDCNN	density	Regression	3.067	2.230
FPNCNN	masks	Regression	2.817	1.727
ERDCNN	density + masks	Regression	2.877	2.114
ResNet-34 (MSE)	None	Regression	1.378	1.001
ResNet-50 (MSE)	None	Regression	1.512	1.114
ResNet-101 (MSE)	None	Regression	2.140	1.551
Ours (only ResNet-34 (CE))	None	Classification	**0.400**	**0.040**
Ours (only ResNet-50 (CE))	None	Classification	0.757	0.093
Ours (only ResNet-101 (CE))	None	Classification	0.841	0.098

Exp 2: Random Missing Counts

We perform a three-round experiment to test the effectiveness of DA, in each round, we randomly remove five cell counts along with their images from the training set.

Exp 3: Consecutive Missing Counts

Same setting as Exp2, in each round, we remove five consecutive cell counts along with their images from the training set.

Table2. *Deleted cell counts in Exp 2 & 3.*

Random (Exp. 2)	Consecutive (Exp. 3)
Round 1 14 35 57 66 83	61 66 70 74 78
Round 2 10 31 70 83 91	70 74 78 83 87
Round 3 18 27 44 53 91	83 87 91 96 100

Table3. *An ablation study of methods in Exp 2 & 3.*

Method	Exp. 2		Exp. 3	
	RMSE	MAE	RMSE	MAE
ResNet-34 (CE)	3.045 ± 0.143	1.238 ± 0.056	6.153 ± 1.071	2.575 ± 0.477
DA	2.120 ± 0.105	0.615 ± 0.134	2.335 ± 0.269	0.792 ± 0.078
Ensemble	1.359 ± 0.032	0.556 ± 0.016	1.983 ± 0.454	0.843 ± 0.188
DA+Ensemble	$\mathbf{1.103 \pm 0.041}$	$\mathbf{0.350 \pm 0.031}$	$\mathbf{1.664 \pm 0.275}$	$\mathbf{0.621 \pm 0.083}$

Table4. *Test RMSE and MAE in Exp 2 & 3. The DA and ensemble module are enabled in this experiment.*

Method	Exp. 2		Exp. 3	
	RMSE	MAE	RMSE	MAE
DRDCNN	4.103 ± 0.213	3.405 ± 0.261	4.480 ± 0.484	3.509 ± 0.522
FPNCNN	2.503 ± 0.195	1.902 ± 0.186	3.606 ± 0.702	2.529 ± 0.325
ERDCNN	3.620 ± 1.300	2.845 ± 1.258	3.706 ± 0.495	2.785 ± 0.470
ResNet-34 (MSE)	1.707 ± 0.096	1.242 ± 0.101	2.200 ± 0.399	1.461 ± 0.162
Ours	$\mathbf{1.103 \pm 0.041}$	$\mathbf{0.350 \pm 0.031}$	$\mathbf{1.664 \pm 0.275}$	$\mathbf{0.621 \pm 0.083}$

Exp 4: Reduced Training Set

Randomly delete half of the training images instead of removing all images for a certain cell count to test the effectiveness of the ensemble scheme.

Table5. *Test RMSE and MAE in Exp 4. The DA module is disabled in this experiment.*

Methods	RMSE	MAE
DRDCNN	16.139 ± 22.767	13.669 ± 19.772
FPNCNN	3.342 ± 0.516	2.556 ± 0.381
ERDCNN	3.531 ± 0.440	2.715 ± 0.374
ResNet-34 (MSE)	2.863 ± 0.614	2.124 ± 0.458
Ours (only ResNet-34 (CE))	2.753 ± 0.274	1.017 ± 0.224
Ours (Ensemble)	$\mathbf{1.969 \pm 0.348}$	$\mathbf{0.868 \pm 0.196}$

Reference

1. Liu, Qian, et al. "A novel convolutional regression network for cell counting." *2019 IEEE 7th International Conference on Bioinformatics and Computational Biology (ICBCB)*. IEEE, 2019.
2. Hernández, Carlos X., Mohammad M. Sultan, and Vijay S. Pande. "Using deep learning for segmentation and counting within microscopy data." *arXiv preprint arXiv:1802.10548* (2018).
3. Liu, Qian, et al. "Automated counting of cancer cells by ensembling deep features." *Cells* 8.9 (2019): 1019.
4. Xue, Yao, et al. "Cell counting by regression using convolutional neural network." *European Conference on Computer Vision*. Springer, Cham, 2016.