Circulating Tumor Enumeration using Deep Learning

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Abstract: Cancer is the third most killer disease just after infectious and cardiovascular diseases. Existing cancer treatment methods vary among patients based on the type and stage of tumor development. Treatment modalities such as chemotherapy, surgery and radiation are successful when the disease is detected early and regularly monitored. Enumeration and detection of Circulating Tumor Cells (CTC's) is a key monitoring method which involves identification of cancer related substances known as tumor markers which are excreted by primary tumors into patient's blood. The presence, absence or number of CTC's in blood can be used as treatment metric indicator. As such, the metric can be used to evaluate patient's disease progression and determine effectiveness of a treatment option a patient is subjected to. In this paper, we present a deep learning model based on Convolutional Neural Network which learns and enumerates CTC's from stained image samples. With no human intervention, the model learns the best set of representations to enumerate CTC's.

1 INTRODUCTION

Cancer is a disease occurring as a result of genetic mutation or abnormal changes in the genes responsible for regulating the growth of body cells. The cells gain ability to keep dividing without control, producing more cells and forming a tumor (Ferlay et al., 2010). The abnormal cells infiltrates healthy ones and spreads throughout the body.

According to Fitzmaurice et al. (2015) cancer caused more than 8 million deaths globally in the year 2013. The cancer death burden have been recorded in both first and third world countries though in an unequal measure. Cancer cases have been exacerbated by unique geographical, cultural and demographic factors such as aging population and increased predisposition to cancer causing conditions such as smoking, being overweight and physical inactivity.

The death figures in first world countries are relatively lower as compared to third world countries. Developing nations have contributed to 57% of cancer total cases. This percentage accounts for approximately 65% of related deaths worldwide (Torre et al., 2015). The higher figures can be attributed to inability to access proper medication and lack of regular monitoring.

Today, different types of cancer are treatable if detected early. Standard medical detection modalities include Radiography, Magnetic Resonance Imaging (MRI), Computer Tomography (CT) and Ultrasound. After cancer detection process, a patient is subjected to appropriate treatment option based on stage and type of tumor. For a given patient, medical surgery or radiation would apply, for others chemotherapy, while hormone therapy for other subjects.

During treatment, a patient must be constantly monitored to determine effectiveness of treatment method they are subjected to. This can be achieved through medical processes such as biopsy or liquid biopsy. Biopsy is a medical procedure which can be used to detect tumor related substances known as Circulating Tumor Cells (CTC's). The process involves identification of CTC's in a raw body tissues. In contrast, liquid biopsy involves detection of the CTC's in blood after staining the sample.

Tumors excretes CTC's into patient's blood. During treatment, the presence, absence or the number CTC's in blood can be used as patient's progress metric indicator to evaluate how the subject is responding to treatment. The metric also determines the effectivenesses of treatment option one is enrolled in. Besides monitoring cancer treatment response and determining the effectiveness of a treatment method, biopsies are also key in assessing the cancer recurrence and progression (Crowley et al., 2013).

In this research work, we present a CTC enumeration model. We show how Convolutional Neural Network (ConvNet) can be used to learn intricate features for enumerating CTC's in a given stained sample image.

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An image sample may contain more than one CTC or none. Based on this, we developed an algorithm to locate CTC's and generate training samples from an input image. We also show that certain models are incapable of learning abstract representative details as opposed to the preferred model.

2 RELATED WORK

Application of learning models in cancer management have been presented by many researchers. Simple and classical learning approaches such as decision trees have been used in detection of common Circulating Tumors variations such as Cytokeratin, Apoptic and Debris. CTC's are extracted following standard procedures such as fictionalized and structured medical wire, Epithelial Cell Adhesion Molecule, density gradient centrifugation or membrane filtration. Scholtens et al. (2012) used Random Forests and Decision Trees to classify CTC's. The target labels were based on different CTC classes such as Apoptic CTC, CTC debris, Leukocytes and Debris.

In another study, Svensson et al. (2014) presented how Naive Bayes classifier and Generative Mixture Model can be used to detect CTC's in stained blood sample. Cells were collected using fictionalized medical wire and thereafter stained. The intensity of RGB (Red, Green, Blue) values of the resulting sample was then used as the input features to the model. The classifier learned to discriminate an instance given the predefined CTC's class.

Aside from classical learning approaches such as Decision Trees, Naive Bayes among other algorithms, deep learning models have also been applied. This learning methodology have led to much better results in image detection and recognition. According to LeCun et al. (2015) deep learning methods have improved the state-of-the-art visual object recognition and detection. This learning approach can automatically discover best set of features to represent an instance. This unique characterization has contributed to unprecedented success studies in the field of image recognition.

Mao et al. (2016) proposed use of Deep Convolutional Neural Network, a deep learning model, to detect Circulating Tumor Cells. The model automatically learned the best set of features used to classify a sample as either having CTC or not. They demonstrated that automated feature discovery using the learning model led to much better results than hand crafted. There was a significant variation in performance when their proposed model was compared to the classical ones such as Support Vector Machines with manually engineered input features.

Wang et al. (2016) also employed a similar learning technique in detection of tumor recurrence yielding state of the art results. In the work, deep learning model developed was aimed at predicting the extent of cancer spread to the other parts of the body.

3 METHODOLOGY

3.1 Dataset

Data used in this research work was secondary data. It was provided by a PhD candidate in Computer Science from Missouri University of Science and Technology. It is the same dataset used in (Mao et al., 2016). The original work was based on classification of CTC's as opposed to enumeration which this paper focuses on. Figure 1 and Figure 2 shows sample images. Figure 1 is a sample of microscopy image. Figure 2 on the other hand is a sample fluorescence image. The latter is used to locate the exact location of the CTC's in the former. Every microscopy image has a corresponding fluorescence image showing CTC's locations.

We developed an algorithm to extract the regions of interest (ROI) to generate both the positive (with CTC) and negative (without CTC) training and testing sets. A total of 1904 training samples were generated. Out of this, 952 were positive and 952 negative. Every sample had a corresponding target label equivalent to the number of CTC's contained. Positive samples had N CTC's and 0 for negative instances. 80% of the dataset was used for training and the remaining 20% for testing the model.

3.2 Cropping Algorithm

We developed an algorithm to extract 40 by 40 the regions of interest (ROI). It accepts x and y coordinates of CTC location in the fluorescence image and a corresponding microscopy image. Following this, 20-pixel coordinate location to the left of x and to the right are marked and saved. The same process also applies for the y coordinate. Random x coordinate within image width range is generated and another random y within image height range. These random x, y values represent the location of new negative sample instance. If these randomly generated coordinates range do not overlap with CTC location bounds then positive and negative sample is cropped out from the 1600x1600 input image and returned. The algorithm have been summarized by Listing 1.

```
crop_sample(xpixel, ypixel, image)
 counter  <- 1
 initialise xaxes[], yaxes[]
 initialise samples[]
 xaxes[0] <- xpixel
 yaxes[0] <- ypixel
 for left_coordinates and
 right_coordinates do:
   while counter < 21
     xaxes[counter+1] = xpixel + counter
     yaxes[counter+1] = ypixel + counter
     counter <- counter+1
 randomx <- random{0,1580}
 randomy <- random{0,1280}
 if x intersection xaxes is empty and
 y intersection yaxes is empty
  samples[0] <- crop(xpixel,ypixel)
  samples[1] <- crop(x,y)
return samples
```

Listing 1: The Cropping Algorithm.

Figure 1: 1600x1600 A microscopy image containing CTC's.

Figure 2: 1600x1600 Fluorescence image depicting location of CTC's in Figure 1.

3.3 Training Set Generation

Microscopy images contains CTC's within specific locations. These images were all converted to one channel from RGB. Fluorescence images on the other hand shows the location or coordinates of CTC's. All images in this set were converted to gray scale and binary thresholded. Thresholding helped to accurately locate the Circulating Tumors Cells.

Binary thresholding involves converting all the image pixels to either of the two predefined values; black or white (Gonzalez and Woods, 2002). To achieve this, pixel intensity values less than 240 were set to 0 and 255 otherwise for the florescence images.

All fluorescence image samples were thresholded using similar function and x, y coordinates of white pixels then mapped to the corresponding one channel microscopy image. This process was then followed by cropping out a positive and negative samples of dimension 40 by 40. The negative set was generated by randomly generating x, y coordinates, checking for the overlap with the positive sample bounds and cropping then cropping out. The feature set was created in this fashion repeating the process over all the images. The target labels for the negative samples were set 0 and N; number of white pixels for the positive instances. Resulting feature sets and targets were persistently stored. Table 1 summarizes the dimensions of training and testing dataset dimensions. Cropped positive and negative samples exhibit different patterns. A high-level depiction of this variation is illustrated by Figure 3 and Figure 4. Figure 3 shows sample positive while Figure 4 represents negative both randomly sampled from training dataset.

Table 1: A summary of training and testing dataset dimensions.

Dataset	Dimension
X_train	(1523, 40, 40)
y_train	(1523, 1)
X_test	(381, 40, 40)
y_test	(381, 1)

3.4 Model Architecture

Convolutional Neural Network (ConvNet) model was used to enumerate the CTC's given the feature sets and labels. The model architecture is captured by Figure 5. The network predicts number of CTC's given an image sample thus a regression task. The ConvNet is composed of Convolution - Max Pooling - Convolution - Max Pooling - Fully Connected Layer (FC1) - Fully Connected Layer (FC2). The architecture is shown by Figure 5 During forward propagation 40 by

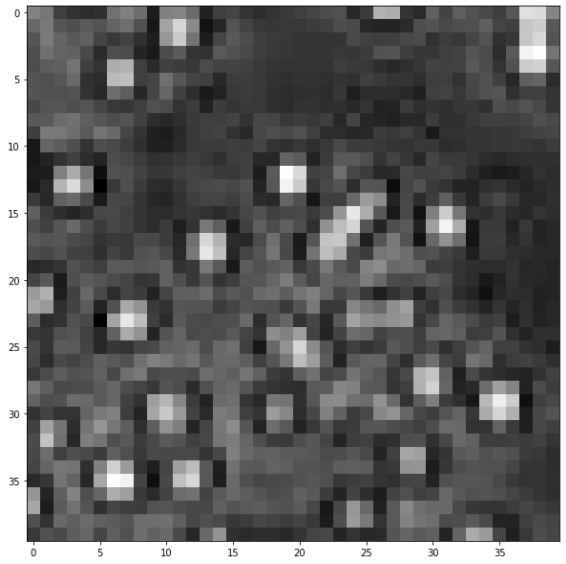


Figure 3: Cropped 40x40 negative sample with 0 CTC's.

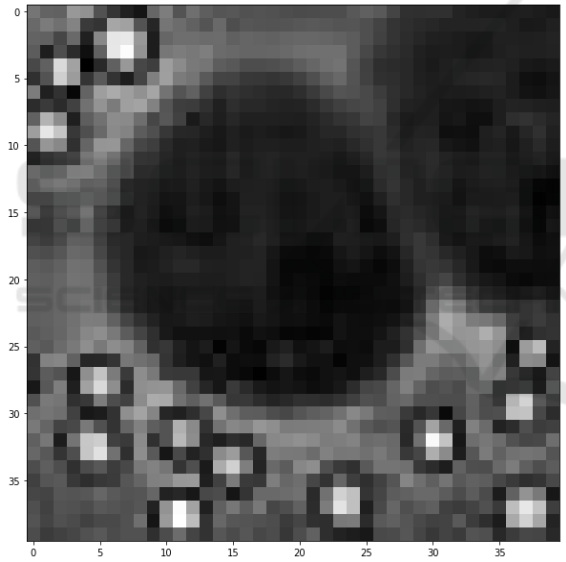


Figure 4: Cropped 40x40 positive sample with given number of CTC's.

40 input was fed into the network. A kernel of shape 5*5 was used to convolve the input image with a slide of 1 resulting in 8 feature maps. The convolution step was then followed by Rectified Linear Units activation mathematically formulated by equation 1. The activation step was then followed by 2*2 max pooling which samples out set of values based on maximum value. The second convolution layer resulted in 16 feature maps which were then flattened and connected two Fully Connected layers.

$$f(x) = \max(0, x) \tag{1}$$

The last Fully Connected layer of neurons was connected to one output neuron which produced a continuous value. This value was then compared to the actual target label and the difference computed resulting in an error, E. The value of E is the mean squared error formulated by equation 3. The error value was back propagated and used to adjust the network weights. The model was trained over 5,000 iterations using backpropagation with batch gradient descent. The gradient descent update rule for weights W and bias b is formulated the by equation 2.

$$w_i := w_i - \alpha \frac{\partial}{\partial w_i} E \quad b_i := b_i - \alpha \frac{\partial}{\partial b_i} E \tag{2}$$

Besides Convolutional Neural Network, Multilayer Neural Network (MLP) and Linear Regression could have solved the problem. These two models were also experimented on the same training and test set. Google cloud platform in relation to software specifications in table 2 was used for the experimentation.

Table 2: Development Environment.

Platform	Application	Version
Python 2.7	Keras	2.1.1
	Tensorflow	1.4.0
	Numpy	1.14.1
	OpenCV	3.3.0

4 RESULTS

Predicting number of CTC's is a regression task. Three different models were experimented. The first model was Convolutional Neural Network (ConvNet) based on the model architecture represented by Figure 5. The second one was a three layer Multilayer Perceptron (MLP) with 1600 neurons in the two hidden layers and one neuron in the output layer. Last model experimented was Linear Regression trained with stochastic gradient descent over 5000 iterations.

Learning with Multi Layer perceptron is relatively algorithmically expensive with many layers due to exponential increase in the number of weights (Nielsen, 2015). Linear Regression on the other hand, though not algorithmically expensive, does not perform well as compared to MLP and ConvNet. The results of all the models based on training and testing errors have been summarized by Table 3. The variation of loss value with respect to number of epochs have also been captured by Figure 6. The ConvNet model was the best performer on both testing and training feature instances. The performance was benchmarked on root mean squared error formulated by equation 3. It sums

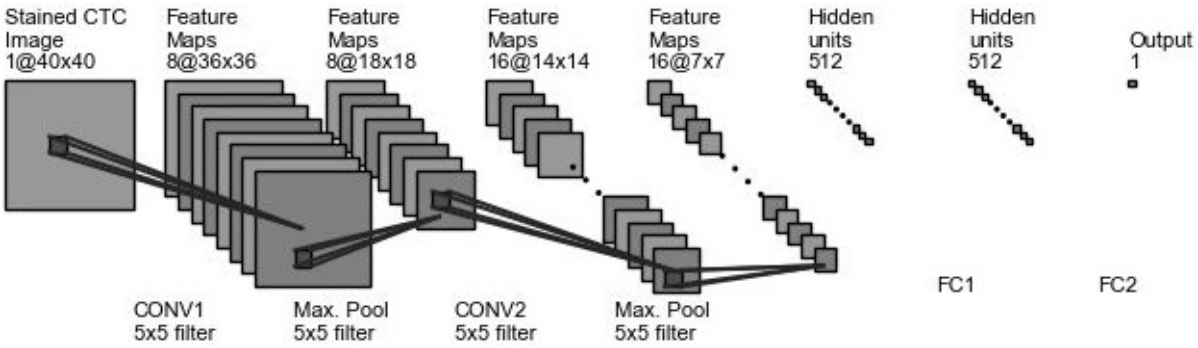


Figure 5: The ConvNet Architecture.

Table 3: Models Results.

Model	Train Error	Test Error	Iterations
Multi Layer Perceptron	0.0364	6.1127	5,000
Convolutional Neural Network	0.0295	4.2822	5,000
Linear Regression	3.475	17.2613	5,000

the difference between predicted ($\hat{y}^{(i)}$) and actual values ($y^{(i)}$).

$$\sum_{i=1}^{m} \left(\hat{y}^{(i)} - y^{(i)} \right) \tag{3}$$

5 DISCUSSIONS

In this research, Convolutional Neural Network outperformed both MLP and Linear regression with a Root Mean Squared Error (RMSE) margin of 0.0069 and 3.4455 respectively on training error, and 1.8305 and 12.9791 respectively on test error. This performance can be attributed to the fact that deep learning models are capable of learning the best set of representative features automatically. The learned feature set is weighted and used to enumerate CTC's given a new sample instance.

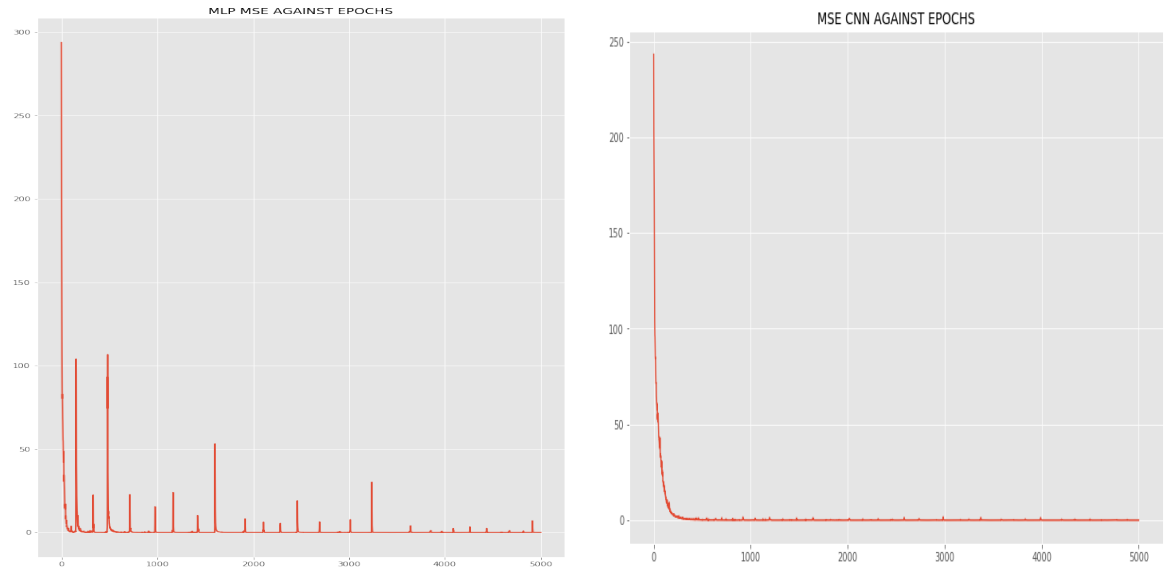
Linear Regression model was trained on all the pixel intensity values of the input image. It was the worst performer just after MLP. The Multilayer layer perceptron model outperformed Linear Regression with a variation of 3.4386 on train error and 11.146 on the test error. From this performance record it can be deduced that not all pixel intensity values are equally informative.

Manually generated features such as RGB histogram or pixel intensity values though can represent an instance, may require the expert to specify the right set of features or extensive feature engineering techniques thus expensive. Using all image pixel values is an assumption that all pixel intensities are representative of an instance.

In contrary, the feature representations can be learned automatically, on a low dimensional space during the training process. This is the core functional and theoretical underpinning of deep learning models such as Convolutional Neural Network. The deep learning adoption was precipitated by the work done by Krizhevsky et al. (2012). In the study, the result obtained halved error value for object recognition during ImageNet competition. This was unprecedented performance record as Convolutional Neural Network model outperformed all other classical simple learning models used before. Following this, other studies have extended the capability of classical ConvNet model leading to state-of-the art results in image recognition (He et al., 2016), large scale image recognition architectures Szegedy et al. (2015) and unprecedented object detection performance Redmon et al. (2016); Ren et al. (2015); Liu et al. (2016).

6 CONCLUSIONS

In this paper, we have presented a learning model which can be used to enumerate Circulating Tumor Cells (CTC's) in a stained image sample. An algorithm which extracts regions of interest was developed and based on the experiments carried out, Convolutional Neural Network outperformed both Linear Regression and classical Multilayer Neural Network architectures. This performance is attributed to the fact that the preferred model had the potent to learn intricate low level representative features on its own. This is a unique characterization which have been attributed to deep learning models such as ConvNet.



(a) MLPLoss (b) ConvNet Loss

Figure 6: MLP and ConvNet loss variations over epochs during training.

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