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Drosophila Heart Recognition System using Convolutional Neural Networks

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by

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Contents

Abstract

In this thesis, we introduced a new method of marking the heart region of *Drosophila* in its different living stages which are known as larva, pupa and adult with convolutional neural networks. The images of *Drosophila* are acquired by a custom optical coherence microscopy (OCM) system in lab, this method of extracting the heart position of the fly obtained by the convolutional neural network in this article is of considerable significance for future research on the fly heart. We will elaborate in the article on how we completed the training and testing of this model and how we acquire the training and testing data of this experiment.

Chapter 1: Introduction

1.1. OCM system

Optical coherence tomography (OCT) is one of the most widely used imaging techniques that measures the interference between a reference beam and a detected beam which is been reflected by the object to the detector[1]. It provides real-time 3D imaging capabilities while providing higher imaging speed and better resolution than conventional imaging technology[2]. OCM technically has advantages of both OCT and confocal , but it has deeper penetration depth compared with traditional OCT.

OCT and OCM are both important tools in bioimaging. They can perform real-time imaging of the biological organs. Research of heart functions on the model of *Drosophila*[3,4] has important implications for the study of human heart.

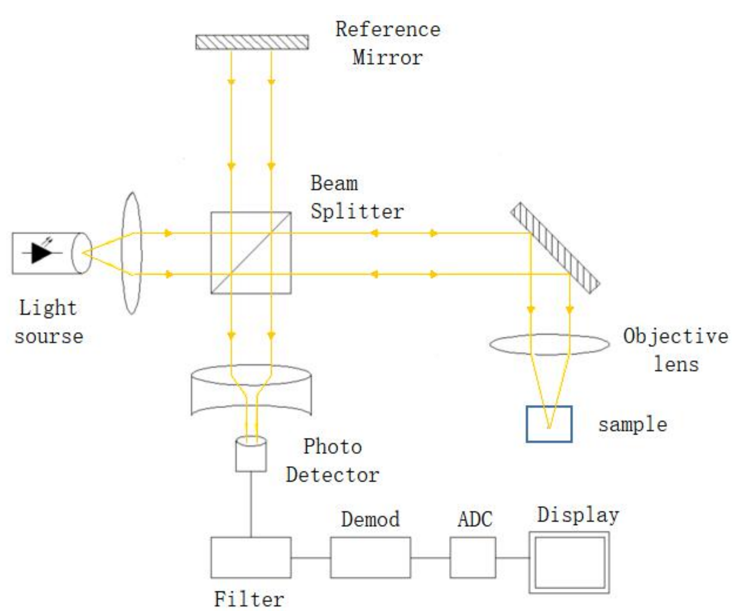

Figure 1- 1. typical single point OCT

As Figure 1-1 presents, the theoretical basis for optical coherence tomography system is low-coherence interferometry, the light from light source is divided into two arms by beam splitter, light reflected by the beam splitter are reflected again by the reference mirror, and this arm is called the reference arm, light refracted will reach the sample and then be reflected back to the beam splitter, this arm is called sample arm. Since the reference arm and sample arm has same optical distance, by collecting the interference pattern of both arms we get time-domained OCT.

1.2. *Drosophila melanogaster*

Drosophila is an important model system for biology and genetics, especially in the study of human heart functions and diseases. Most of the human pathogenic genes also have the same expression in Drosophila[3,4]. Genes that control cardiac specification and morphogenesis are very similar in Drosophila and humans. So it is believed that results of genetic studies on the Drosophila heart may also apply to humans.

1.3. Convolutional Neural Networks

Recently, convolutional neural networks(CNN) is widely used in solving many problems which allows the models to be separated into multiple processing layers[5,6], with backpropagation algorithm, machine could calculate the representation in the new layer from the representation transmitted from the former layer[7,8]. as shown in figure 1-2.

Convolutional neural networks are in some way similar to the ordinary neural networks in deep learning[9], they contain different neurons(layers) with inputs and outputs, there is a loss on the last connected neuron which can be presented as loss function to measure the feed back of the system. However, Convolutional neural networks only use images as input or output, the calculation of date based on pixels of the input images[5].

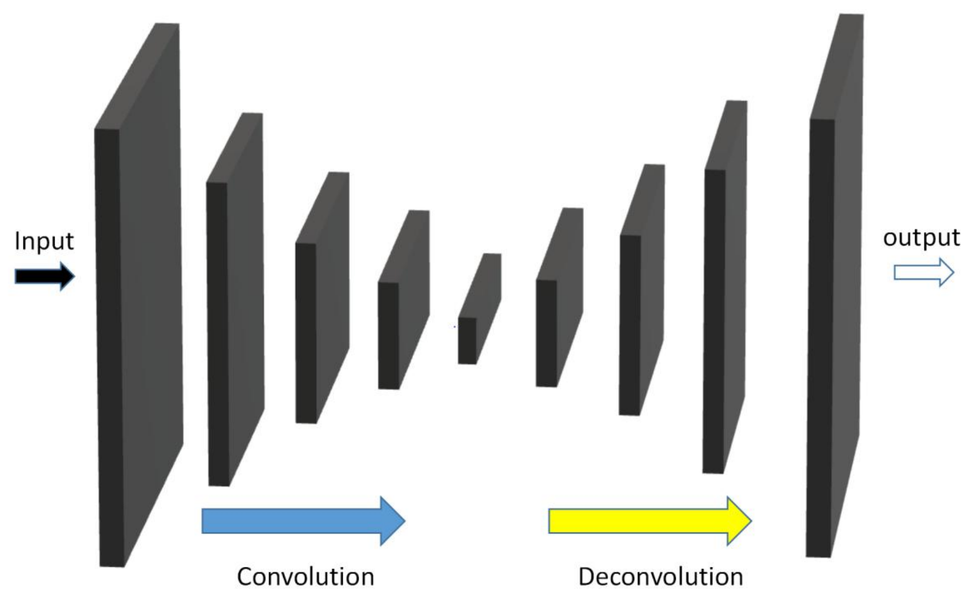

Figure 1- 2. Layers of convolutional neural networks

At the end of the 20th century[9], the structure of convolutional neural networks was first proposed and successfully implemented. With the development of GPU technology, the application of convolutional neural networks has become more and more widespread. Many studies have focused on convolutional neural networks, and many applications have been based on this method[10-12].

To validate CNN's effective prediction of heart position, a standard called IOU(intersection over union) is introduced[13](1.1), which is also know as Jaccard Simularity [14] between the predicted heart region and human marked heart region.

$$\mathrm{sim}(C_1, C_2) = \frac{|C_1 \cap C_2|}{|C_1 \cup C_2|} \tag{1.1}$$

1.4. Motivation

The former prediction model in our lab was based on matlab code without machine learning methods, and the prediction itself is not accurate enough to be used as lab data, so we manually acquired a large amount of post-marked data and used this as a basis to implement the CNN model[15].

The laboratory has ready-made database of heart observations at each stage of *Drosophila*, however it relies on manual work to do the segmentation in researches. The completion of the CNN model training will greatly facilitate the future research on the heart of the fly, including the beating cycle, heart diameter, and area change at a real-time speed[18-21].

Before the CNN model was used to identify the heart of the fly, the laboratory used a segmentation program written in matlab. Figure 1-3 shows the application interface of the program. First we need to select an ROI in the front panel shown in Figure.1-3a and then adjust two parameters in the front panel shown in Figure.1-3b. The two parameters named max threshold and min threshold are adjusted in the illustrated interface. The purpose of adjusting these two parameters is that we need to convert the grayscale image collected by the OCM system into a black and white image that can be processed more easily by the program. Therefore, we directly convert the pixels whose grayscale are less than a certain value to white pixels and pixels whose grayscale greater than a certain value are converted to black pixels.

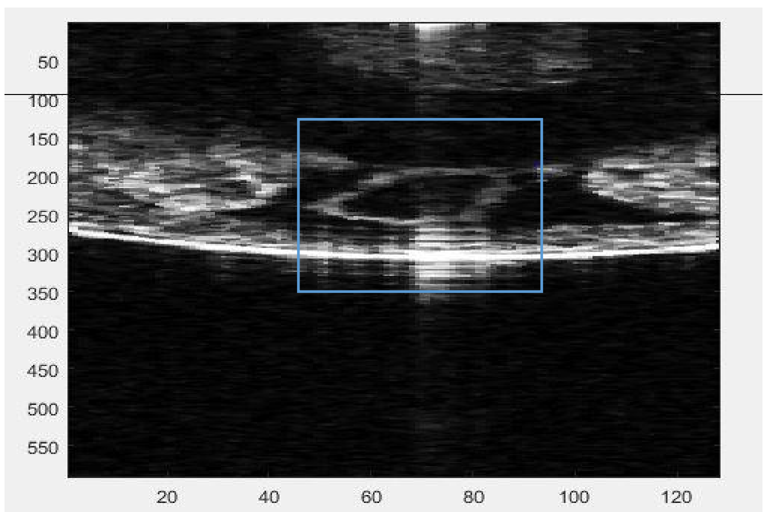

Figure 1- 3a. The first front panel, select the ROI

Figure 1-3b. The second front panel, select two parameters and get preview of the segmentation

Then we need to select a black pixel inside the heart as the starting position, select another black pixel outside the heart as the mark of outside area, and expand area from the starting point until it meets the white boundary pixels to get the heart area.

The core algorithm of the program is to start from a pixel inside the heart and gradually expand outward until it meets the part that is judged as the boundary. For the contaminated area inside the heart formed by the device, when the diameter of area is less than a certain threshold, the system will automatically classify this part as the heart area, and for the discontinuous area of the heart boundary, when the distance between the two boundary points is less than the threshold, the system will automatically stop the expansion of the heart area to identify it as the boundary.

The problem with this algorithm is that it is easy to cause leaks in the determination of the heart region when encountering a blurred border. And the heart is in the process of contraction and diastole it is necessary to accurately determine the position of the center of the heart and calibrate it every time. The efficiency and accuracy of the program needs to be improved, which is one of the reasons we introduced the CNN model.

1.5. Thesis organization

This article will be divided into five parts. The first part is the introduction to the convolutional neural networks and our motivation. In the second part we will explain how we acquire and filter the training and test data to get a better IOU. The third part will introduce the structure of the convolutional neural networks and the training and testing process. The fourth part shows the result of well trained model, the fifth part is the significance of this experiment and our outlook for the future.

Chapter 2: Data collection

2.1. Unprocessed dataset

According to a previous laboratory study of the heartbeat of the fly[22], the laboratory has collected OCM images of the heartbeat data of the fly, each set of images completely records several beat cycles of *Drosophila* hearts. For one image set there are 4096 images of one *Drosophila* heart taken continuously with OCM system, it is showed in Figure 2-1 one sample frame for each stage of *Drosophila* heart.

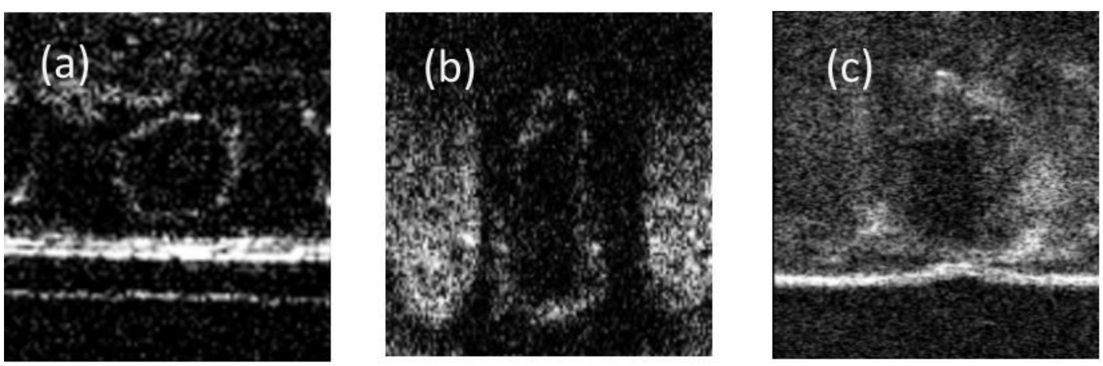

Figure 2- 1. Different stages of *Drosophila* heart
(a)larva (b)pupa (c)adult

The images of *Drosophila* hearts are acquired by a custom optical coherence microscopy (OCM) system[2,16,17], with a central wavelength of ~800 nm and a bandwidth of ~220 nm, and a 2048 pixel line scan camera operating at 20k A-scans/s. The OCM images reach an axial resolution of ~1.5 um and transverse resolution of ~3.9 um. The *Drosophila* hearts has three different stages which are larva, pupa and adult. We select 500 relatively clear continuous images from a set of 4096 images of the same dataset as one test sample and use these samples(20 each for the larva and pupa stage and 15 for the adult stage) to train the CNN model separately. With the increase in number of datasets and particularly different heart shapes added, the accuracy of the CNN prediction model increases. After the training, we need more datasets(1000 images) that has not been model-trained as test data, another 1000 images are required for cross validation.

Our task is to select 100-500 images in each dataset with clear heart locations and manually mark the position of the heart as ground truth, which means we need to mark the boundaries of the heart and make some guesses about some of the blurred areas of the borders to form a closed area that divides the entire image into two parts.

In the figure, the white part is the boundary of the heart of the fly, the black part inside the boundary is considered to be the *Drosophila* heart which is our region of interest(ROI)[23]. To improve the accuracy of the boundary recognition, for a group of original images (128*690*4096), we will first reduce the size of the image to 128*128[15], and make the heart of the fly basically appears in the center of image, it also reduces the amount of image processing data. The other reason for this is because the imaging position of the OCM imaging system makes sure the position of heart is fixed in most cases, and it is also possible to avoid the interference of some edge position image blurring with the final heart position prediction.

2.2. Data selection

For most larva and pupa data, the borders of the heart are clear enough. For a short segment of the clear border[24], it can be easily supplemented by human experience, but for adult fly, a part of the data is unsatisfying, as described in Figure 2-2, we selected partially obscure data as training data, the purpose is for improving the generality of the model. In most circumstances, such data has a negative effect on boundary prediction, but for our further research on fuzzy boundaries and further refinement of the model's functionality, these data cannot be completely discarded.

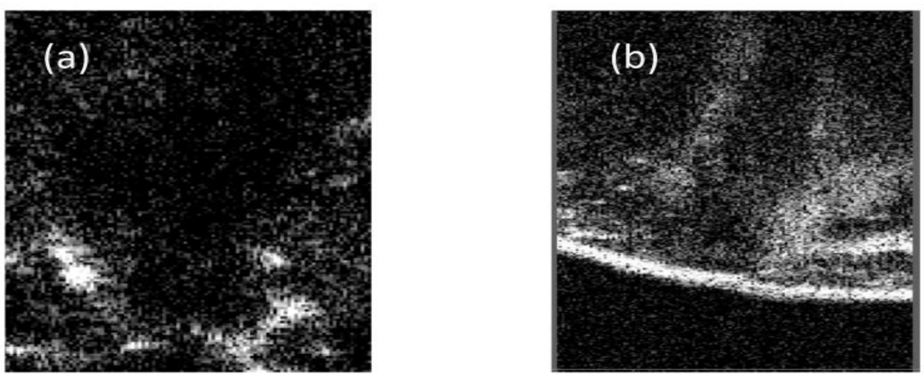

Figure 2- 2. (a)Boundary lost and (b)Strong noise

Initially, we hoped to obtain images with clearest boundary as training label, since it will speed up the training and improve the accuracy. Accordingly for the first few datasets marked, the most distinct images were selected in every single cycle and marked separately regardless of whether they were continuous in time. The errors caused by human factors in the training model could be minimized in this method. The facts prove that when the selected images are used as training labels, the training results which are quite consistent with the artificial mark can be obtained when the images with as clear boundaries are selected as test data.

However,it was soon noticed this method was not correct. For slightly blurred images, the model has obvious problems with the recognition of the boundary[25]. Similarly, for the continuous input of periodic fly heart data, the model can not give satisfying results, and the images we select do not fully represent the average level of all the images acquired with OCM system, so it might not represent the performance of the training system. Therefore, in the following experiments, we selected consecutive 500 images as training labels, accordingly, we used 500 continuous images as test data which has 8-12 heartbeat cycles, which can ensure that each heart's shape and images with different degrees of blurring can be accurately identified.

We also considered some other factors that may affect the accuracy of the final prediction, which must be excluded at the stage of data acquisition. It is initially suspected that different people's definition of the heart's borders may not be the same, and the predicting the blurred location might not be the same, it depends on the researchers’ understanding of the heart shape. To rule out human factors, we tested the data with cross validation with different markers’ data as ground truth. It turns out that the human subjective factor does not affect the final training model after the training data reaches a certain amount[26], similarly, the improvement of training results is gradually stable with the increase of training dataset number.

2.3. Preprocessing

A visualization and analysis software named Amira was used to preprocess the data. First we manually draw the position of the heart boundaries on the image, and then use the python program to divide the image into black and white parts according to the boundary. Since the original image is a black and white image, use black and white to distinguish To indicate the location of the heart is more conducive to subsequent convolutional neural networks calculations.

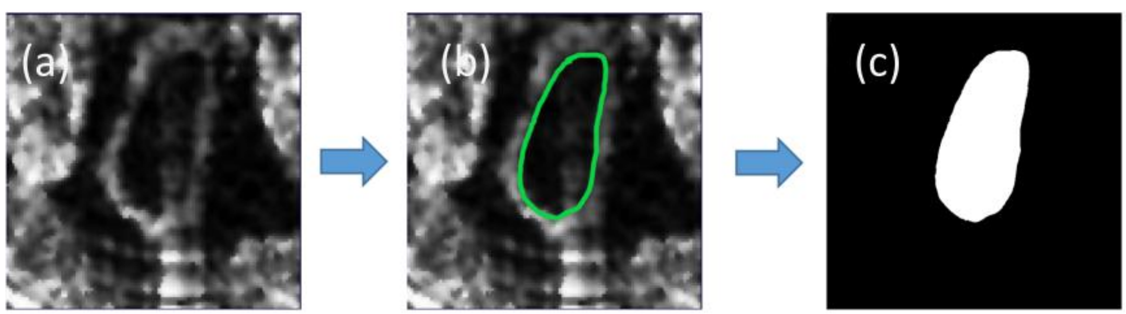

Figure 2- 3. Data Preprocessing (a)unprocessed image (b)marked image(c)input ground truth

This completes the preprocessing of the data, pre-processed data was saved separately for input or ground truth in the model. Through the CNN model we can get the predicted heart position of the fly and compare it with the artificially labeled ground truth to get the required IOU, as the accuracy of the training model[27].

Chapter 3: Methods

3.1. Convolutional neural networks structure

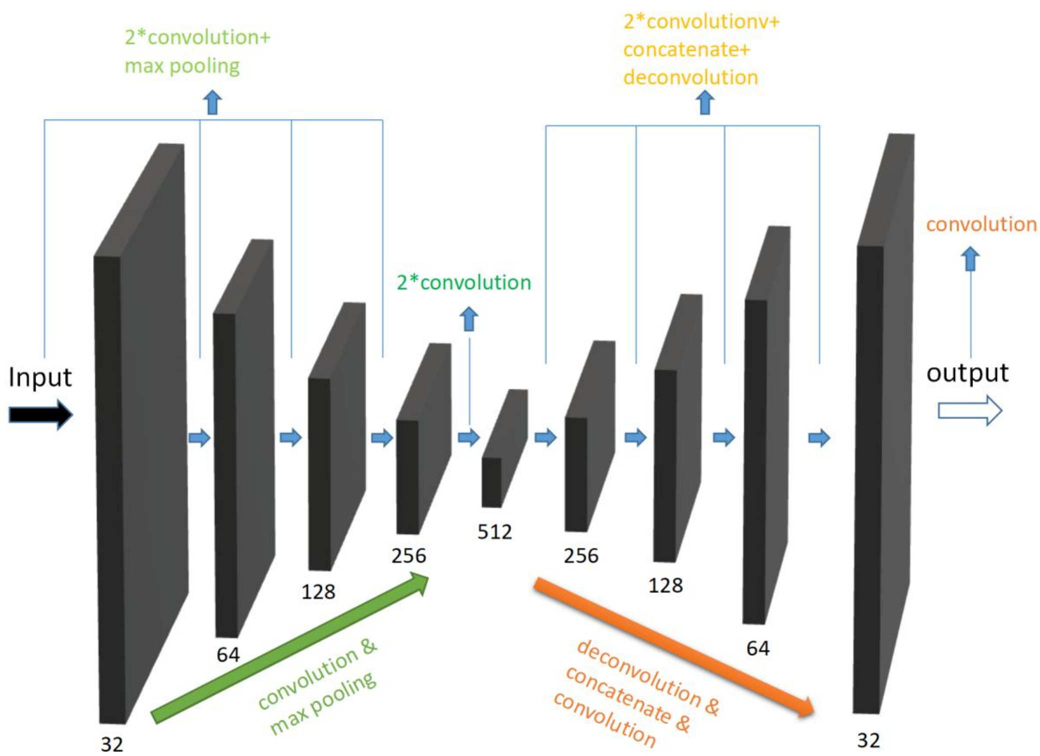

Figure 3- 1. Structure of the CNN

Each neural networks' layer can be represented by a three-dimensional array h * w * d, h is the height of the image and w is the width, and d is used to represent the space occupied by the color of the image, such as an RGB image, then d = 3, respectively, representing the Red, Green, and Blue coefficients of the image. The images we use do not care about the color information. Therefore,in this case d=1, which is used to characterize the grayscale only.

For any input Xij in each layer, there is a computed data Yij in the corresponding position in the next layer and there is the following numerical relationship,in which k is the kernel size, s is the subsampling factor, the function type decides the layer type:

$$y_{ij} = f_{ks}\left(\{x_{si+\delta i, sj+\delta j}\} 0 \leq \delta i, \delta j \leq k\right)_{[15](3.1)}$$

Figure 3-1 shows us a hierarchical structure of the model, which was modified from U-Net structure presented in Figure 3-3[28], it combines a contracting path with an expansive path(which is also encoder and decoder path), the input image size is 128*128(to make sure the output image is complete, the x- and y- size must match each other). The first four left arrows, including the input arrow, consist of repeated two 3x3 convolution layers, in each layer there is also a max pooling layer whose size is 2x2[29] after the Rectified linear unit(ReLU), followed by two standard convolutional layers. For the expansive path, each arrow(from sixth to ninth, marked with orange) contains two convolutional layers, one deconvolution layer and a concatenate operation. Feature maps of corresponding dimensions are connected using concatenate operation to decrease loss. Finally, it is a 1 x 1 convolution to mark the possibility of each pixel to judge if it belongs to the heart area.

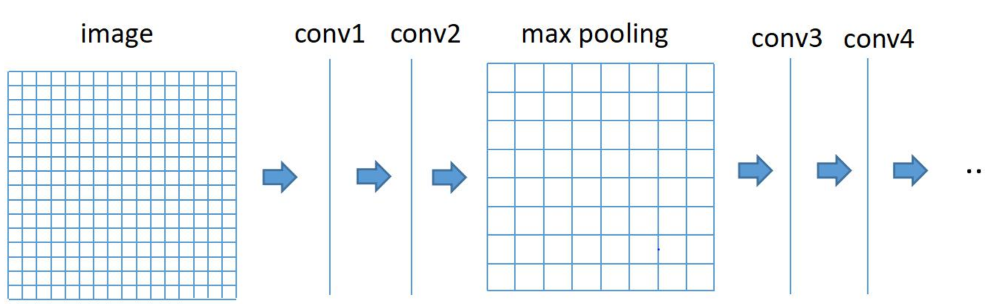

Figure 3- 2. Structure of layers

For each layer we used the following structure in Figure 3-2. Three main types of layers are used to do this segmentation, which are respectfully, Convolutional Layer[30], Pooling Layer[31], and Fully-Connected Layer[32]. The Convolutional layers are used to compute the output neurons of the corresponding input volume, then pass the result to the next layer. The pooling layer is used to down-sample the image of the input layer, reduce its dimension and allow for assumptions to be made about features contained in the sub-regions binned. Fully-Connected layer will compute the class scores, which in this segmentation, judge if one single pixel belongs to heart part of *Drosophila* or not.

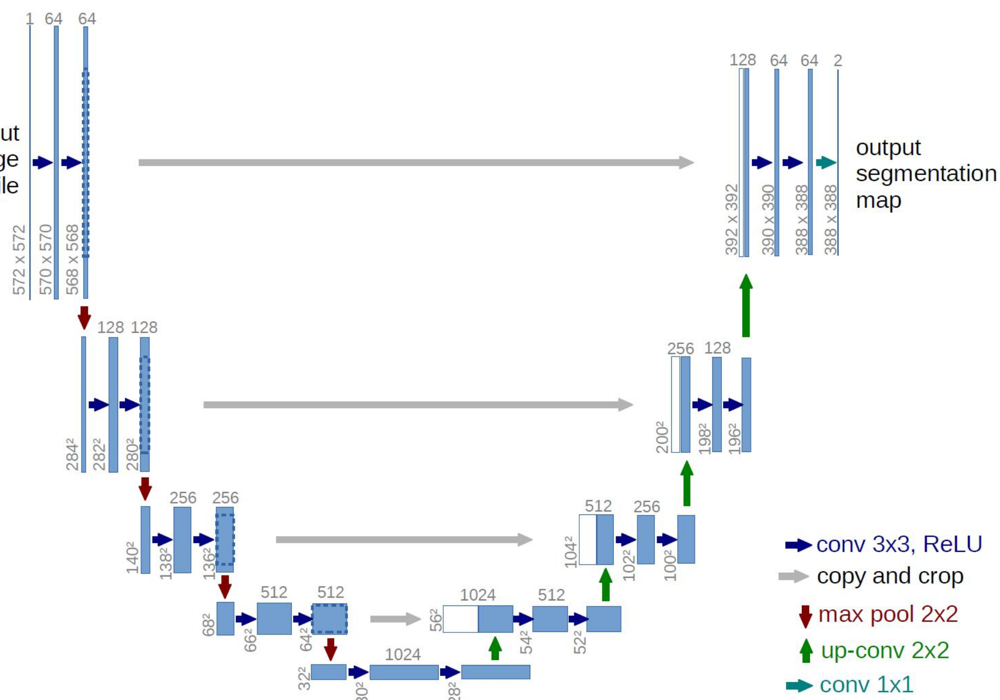

Figure 3- 3. U-net Structure

Figure 3-3 represents the basic U-net architecture (an example with a minimum resolution of 32x32 pixels), which is a simpler version of the model we used in the experiment(our input has a resolution of 128x128 pixels and has more layers), but it clearly describes all the layers of the model we used in the experiment. Each blue bar is connected to a multichannel feature map. The x-y size is in the lower left corner of the bar. The white bar represents the copied feature maps and the definition of arrows are in the lower right corner.

The u-net architecture achieves very good performance in many different biomedical applications. It requires very few training images and takes a relatively short time on the NVidia 1080 GPU to be well trained, in this case it takes less than 25000 images to finish the training and testing task and for every training process it was no more than six hours.

3.2. Training of CNN model

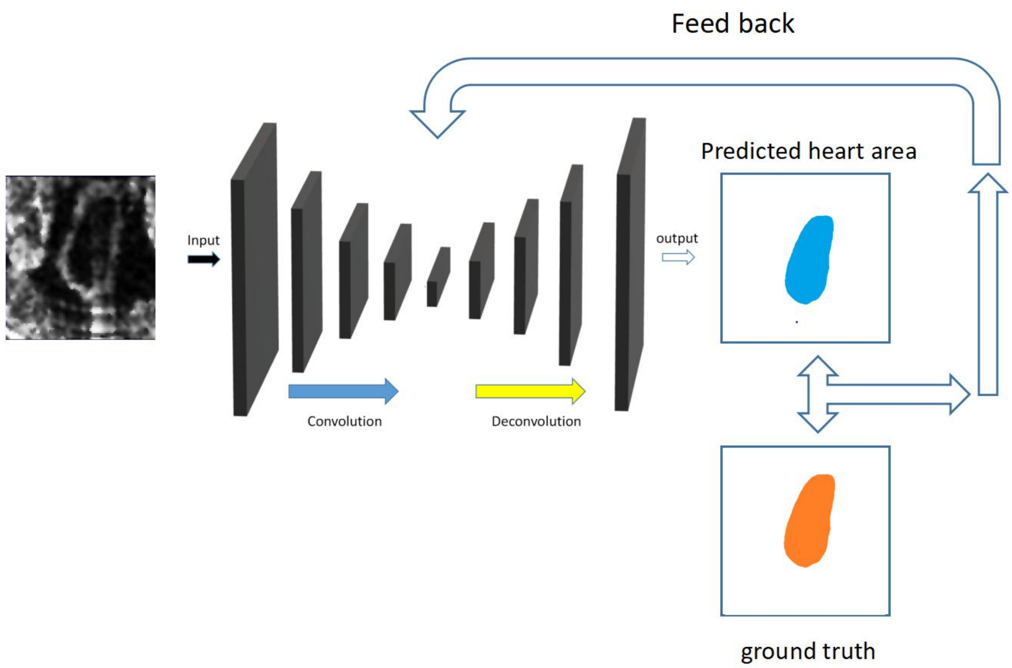

Figure 3- 4. Structure of training process

As shown in Figure 3-4, fly heart image acquired by OCM system was sent to CNN model as input. The model then generates an output image of the segmented result.Then the image generated (shown in blue) was used to and calculate the loss with the ground truth (shown in orange) and fed back to the system to update the weights and bias. This process is repeated 50 times until the stop mechanism is triggered. This reduces the probability of system over-fitting.

3.3. Testing of CNN model

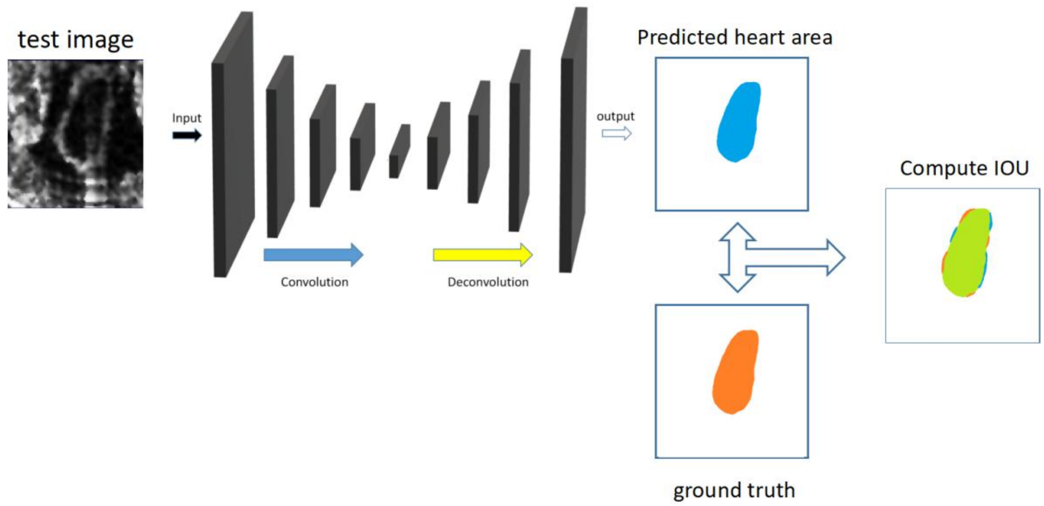

Figure 3- 5. Structure of training process

The test program plan is shown in Figure 3-5. A mature model was obtained after the training process was completed. For the test program, a new OCM image was put into the model, after prediction the result is shown in blue color. The marked heart area(in orange) of the test image is compared with the test result to calculate the IOU to determine the performance of the model. Once the system is well-trained, the test procedure can be used for cardiac segmentation and other data detection of other obtained fly heart images.

3.4 Cross validation of CNN model

In this experiment, K-fold cross-validation was used. Among the 25000 original images, 1000 of them were selected as cross validation objects. The subsample(1000 images) was retained as the data of the validation model, and the other samples were used for training[33]. The cross-validation is repeated 25 times, each sub-sample is verified to obtain a single estimate.The model is retrained every time to ensure that there is no over-fitting.

Chapter 4: Results

4.1. Prediction results of CNN model

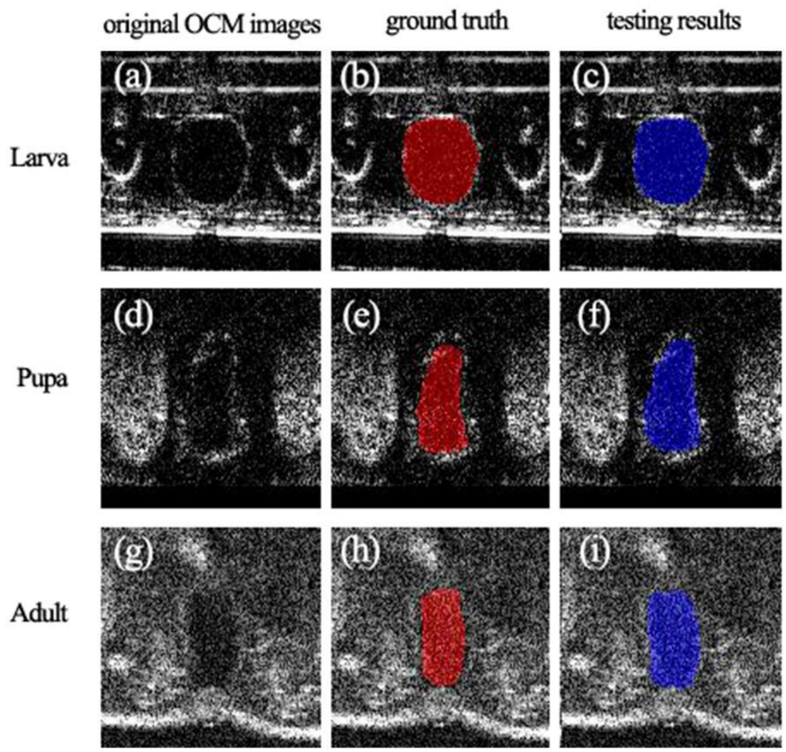

Figure 4- 1. The comparison of original images ground truth and testing

NVDIA Geforce GTX 1080 GPU with 8GB of memory was used to train CNN model,training for a set of data is complete after a total of 20 trials were conducted. The figure shows the results obtained after the training of the model. It represents the model's predictions for larva, pupa, and adult respectively. The raw input is shown in the left，(a) for larva, (d) for pupa and (g) for adult, and the three images in the second column are

The location of the heart marked manually, ground truth, and the third column of images is the model's prediction of heart position.

OCM images of Larva and pupa are generally clearer than Adult, and borders are easier to identify, some images for adult stage are blurred or have discontinuous heart borders. Overall, the model can accurately predict cardiac regions regardless of cardiac cycle stage or developmental stage.

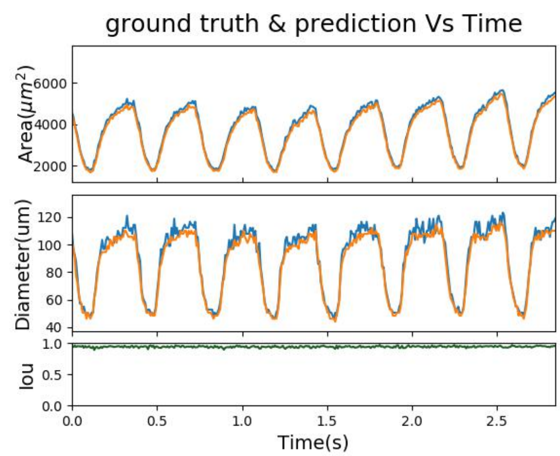

Figure 4- 2a. prediction results with ground truth versus time (larva)

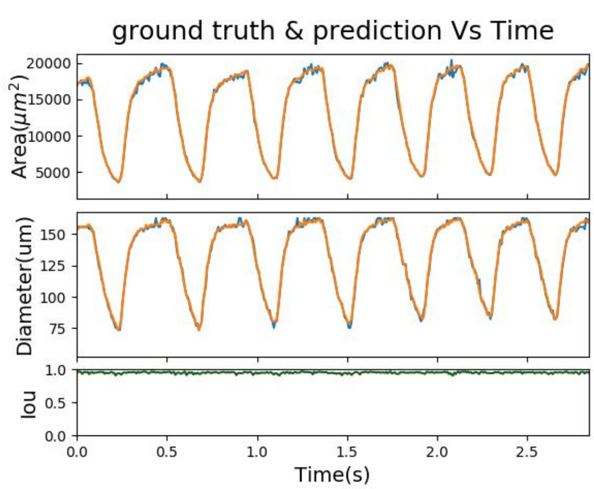

Figure 4- 2b. prediction results with ground truth versus time (pupa)

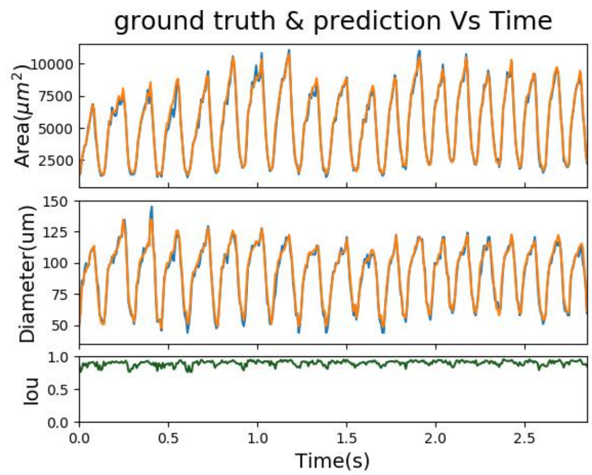

Figure 4- 2c. prediction results with ground truth versus time (adult)

Heart area, diameter, and IOU data for each frame of the test are figured out to quantify the performance of the model and visually demonstrate the ability of the model to predict at different stages. Figure 4-2 shows this result. Figures a, b, and c show the larval, pupal, and adult heart regions, respectively, the diameter of the heart and the changes in the IOU with respect to time. The orange lines represent the hand-drawn ground truth. The blue lines represent the predicted results given by the model after training. On the corresponding time axis, the green lines indicate the IOU data for each frame. Since the data we use is continuous, it can also be intuitively express how each data changes with time.

For larval and pupal data, in the process of systole and diastole, the model prediction results almost completely overlap with the manually collected ground truth, and there is only a small difference between the fully contracted and fully dilated phases, and the overall prediction result is maintained 90% or more, the model can basically replace the experimenter's hand marking and collection of the fly heart data.

For adult flies, the IOU curve has a decreasing trend during the systolic stage, which indicates that the model is not as capable of predicting the heart region of the adult fruit fly during systole stage, but relatively still maintains a high and stable IOU (>75%). It indicates that the result of data on the area and diameter of the fly hearts can be used to characterize heart function and further research and experiments.

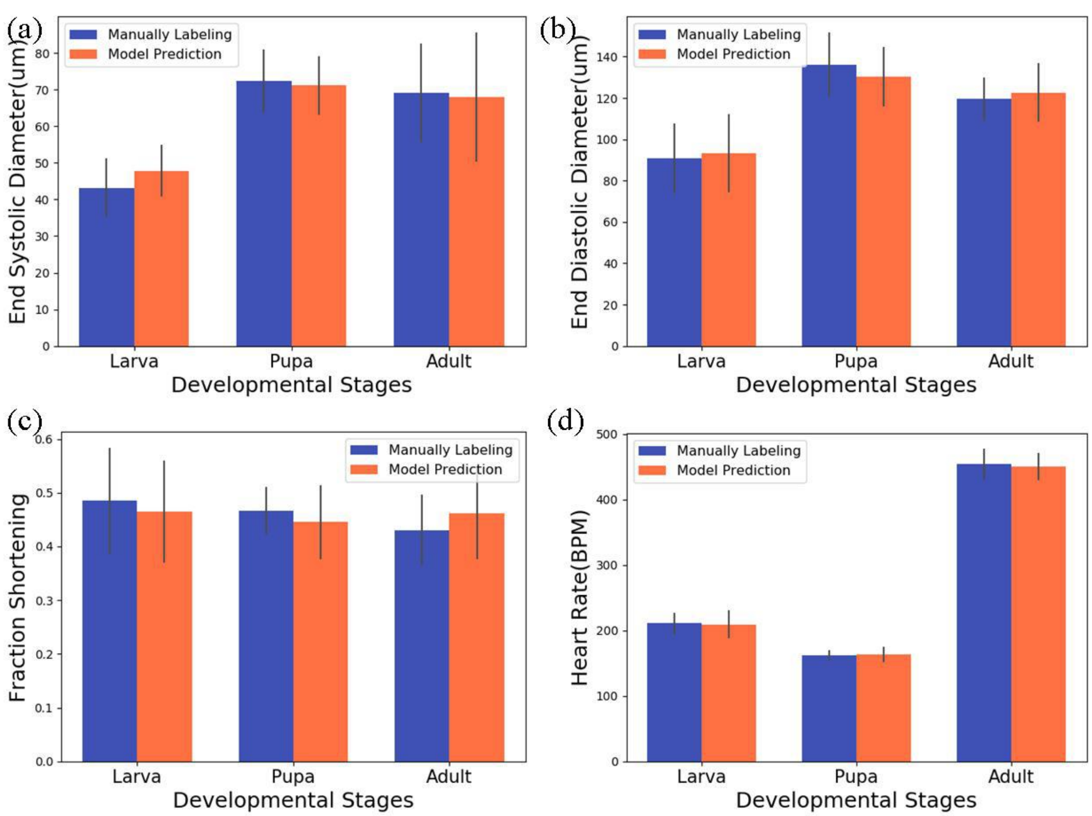

Figure 4- 3. relationship between predicted data and ground truth for four parameters computed

In addition to visually representing the Drosophila heart area and diameter, the model can also provide calculations and analysis of other parameters. The figure 4-3 above shows four calculated parameters as end diastolic diameter (EDD) indicating the diameter during the heart dilation, end systolic diameter (ESD) indicating the diameter during heart contraction, fraction shortening (FS) indicating the diastolic diameter lost in systole, and heart rate (HR)[3,4].

For each figure, the blue bar is generated from manually labeled data, and the orange bar is from predicted data, for each data, comparison of different stages are shown in three different groups, the orange and blue bars indicate average result and the error bars in the middle indicate the standard deviation of each data.

As can be seen in the figure, there is no significant difference between the manually labeled data and the prediction data for each set, which indicates that the prediction model can accurately generate data such as EDD, ESD, FS, HR, and can be used to replace the calculation of artificial marker data.

Chapter 5: Discussions

We used the convolutional neural networks to successfully predict and mark the position of the heart in the fly picture captured by the OCM system. The average IOU predicted with the artificially labeled ground truth can reach approximately 86%, Furthermore, physiological parameters of the fly heartbeat, such as EDD, ESD, FS and HR, can be accurately quantified to characterize *Drosophila* heart function.

For future research, the CNN model we used does not take the continuity of the images as a basis for consideration when making a prediction of the heart position. When a human makes a judgment on the heart position of the fly with naked eyes, the images before and after the frame is often observed, the heart shape is always continuous between neighboring frames. In the future experiments, if when the model judges the boundary of a single frame of image, it also takes the neighboring frames into consideration, the accuracy of the model could be enhanced.

In this project, I was mainly responsible for the collection of training and test data, including marking, screening, and sorting of data. I tried to debug the original matlab program in the laboratory, but did not achieve great results. I learned python programming and how to test and do cross validation for CNN models, which could be of great help to my future work.

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Biography

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