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Analysis of Embryonic Malformations in Zebrafish Larvae

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***Abstract*— Zebrafish larvae have been widely used in testing the effect of chemical substances on the human body. Biological experts have been carrying out these studies manually with a relatively poor productivity. This paper proposes a high-throughput computer vision system to identify and classify embryonic malformations in zebrafish larva. The proposed system, which comprises at least 96-well plates and a flatbed optical scanner, utilizes multi-classification features including shape, intensity, and texture features. A classifier based on artificial neural networks is utilized to address overlapping problems between different classes of the image dataset and thus improving the classification throughput and accuracy. The preliminary results of this pilot study showed a good performance and low recognition error of the classifier in which two classes were recognized according to the rounding shape of the larva.**

***Keywords*— Classification; computer vision; embryo malformation; zebrafish larvae.**

I. INTRODUCTION

These days, the zebrafish (Danio Rerio) has become one of the most popular models used in pharmacological studies [1]. Biologists use zebrafish in their experiments instead of other animals for several reasons including [2], [3]: (i) it can propagate over the year not during a specific time, (ii) the time period between fertilization to hatching is short (72 hours), and (iii) the transparent shape of its body helps biologists in their observations. As the experiments that are done by biologists typically take considerable time in tracking and monitoring using manual observations [4], [5], automatic tracking systems have emerged to help the biologists in making their experiments faster and more accurate.

Zebrafish analysis is divided into many types of automated systems such as heart beat detection [6], [7], monitoring the embryogenesis of cells [8], [9], land marks and tissue recognition [10], [11] and monitoring the behaviour of the adult fish [12], [13]. Tools used by researchers for imaging include confocal [14], [15] or multi-photon laser scanning microscopy that generates three-dimensional time lapse 3D+t imaging and the other is the selective plane illumination microscopy [16], [17].

Numerous technologies and procedures have been proposed for high-throughput and high-content screening [6], [7], [11], [18]. For example, researchers used microscopy imaging as imaging data requirements in many ways to determine some important parameters for high-throughput assays such as the resolution of purchased images, the field of view, the SNR, and the survival of specimens during the assay. However, these technologies and procedures are still facing several challenges with high-content image data either for quality, annotation or storage.

Recognising two classes of the zebrafish embryos was proposed in [19], where the authors designed a system in which the live and dead embryos have been detected automatically. This process came after treating the eggs by adding some chemical substances. In [20], the authors proposed a classifier based on tree learning algorithm for evaluating malformations of the larvae during their growth that are caused by adding chemical substances. They used supervised learning method with image processing to identify only two types of the malformations, edema and curved tail. This work was then extended in [21] through adding other classes to the previous system including hemostasis, necrossed yolk sac, edema, and tail malformations. They used a 6-well plate and a classification model similar to that reported in [20]. Computer-vision systems for tracking and monitoring zabrafish larva have also been of interest in recent years where an electrical stimulator [21], automatic pattern detection method for behavioral analysis [22], and occurrence density index for behavior classification of zebrafish larvae [23] were reported.

According to numerous studies, however, designing of an automated vision system for classifying phenotypes of zebrafish larvae is still a big challenge. In addition, design a high-throughput and accurate classification system to classify the malformation classes has become a critical requirement to help biologists in their pharmacological and toxicological studies that typically take long observation periods prior to making appropriate decisions.

In this paper a high-throughput data acquisition and classification system is proposed to identify and classify malformations in embryos of zebrafish larvae. The captured images are pre-processed and analysed using an improved classifying technique.

The remainder of this paper is organised as follows. Section II overviews the proposed system, Section III discusses the proposed methods and materials, Section IV presents and discusses some initial results, and finally the work is concluded in Section V.

II. SYSTEM OVERVIEW

There are two parts of the proposed system: the hardware part that consists of the biological tools including the well plates and the capturing device, and the software part that is presented by the data analysing and implementing to be used in the classifier model. The analysis of images was done using several techniques

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including enhancement the images in such suitable form then extract the images features before deciding the most important ones to be used in the classifier model with high performance and low recognition error. Finally, the status report is presented to the user in which the collected images of larvae are recognised and classified as in Fig.1.

III. Materials and Methods

A. Dataset

The proposed system will have two sources of the zebra fish embryo images that should be for the whole body of the larva with under 72hpf (hour post fertilization) age before being adult where they are observed easily:

- Image data acquisition system that will be collected automatically using the system hardware in the biological laboratories.
- Images that are used in this study are publicly available [24] with unrestricted use and reproduction.

Types of malformation are presented by abnormality of the embryos tails or in their vessels or the whole body shape. Adding several chemical substances into the surrounded liquid of the larvae causes changes in the tail shape so it goes to be up or down, also the larvae may not be hatched (i.e. chorionic type). Examples of normal and malformed shapes are shown in Fig. 2 and Fig. 3, respectively.

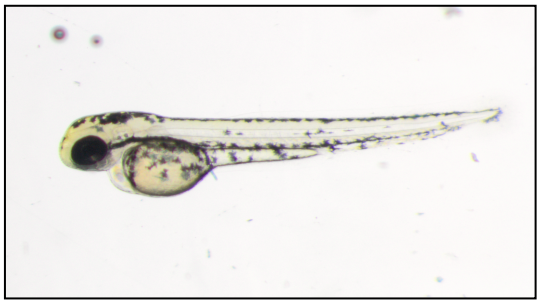


Figure 2. Example of a microscopy image for a dead larva [24]

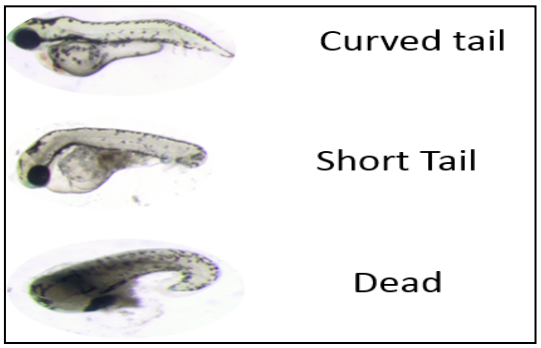


Figure 3. Examples of embryo malformations

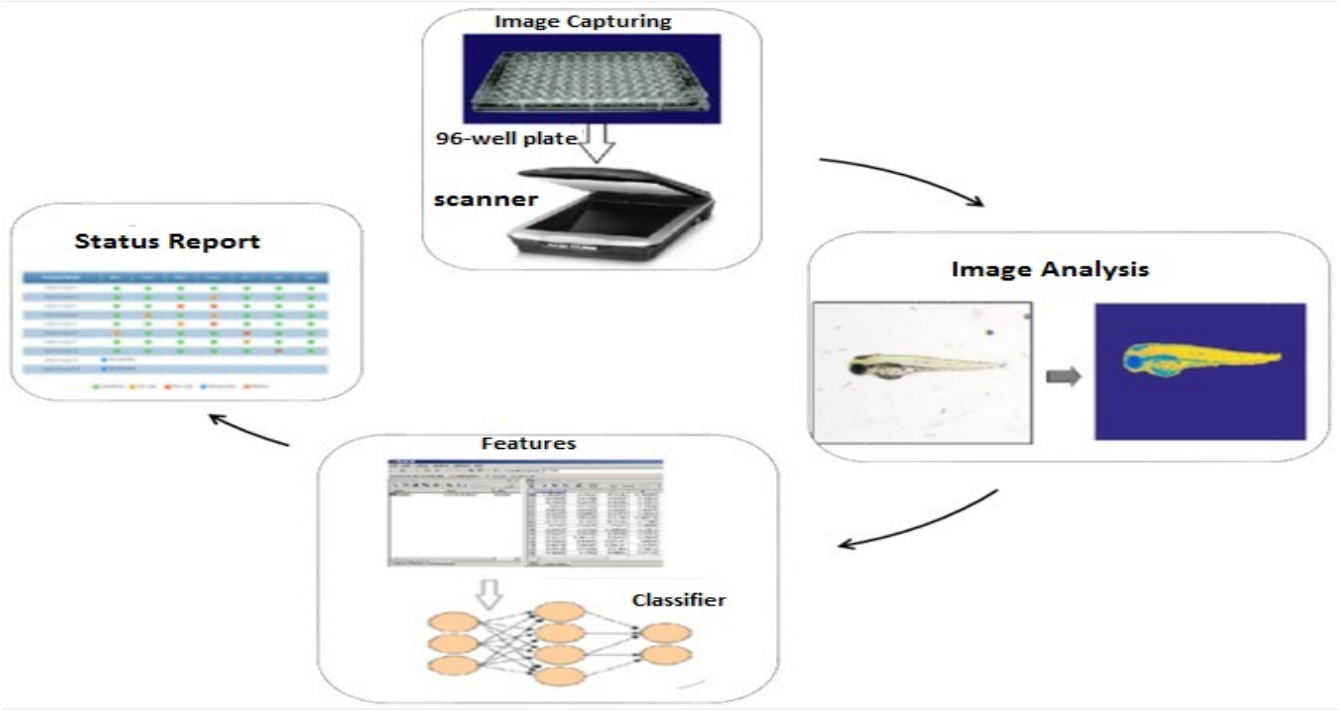


Figure 1. System overview

B. Methodology

The proposed system is divided into several stages: image capturing, pre-processing, feature extraction, feature selection, and classification. These stages are shown in the simplified flowchart of Fig. 4 and are described briefly as follows:

1) Image capturing: The image capturing process will be done automatically and the images will be directly loaded onto the computer. To improve the time-consuming image capturing of the manual process, there are two options that can be outlined briefly as follows:

- Flatbed Optical Scanner: in this method, the well plate(s) will be captured and a multi pictures will be taken in a short period of time which is one second or less for each well of the plate. Fig. 4 shows a block diagram for the proposed methodology. As illustrated, by using scanner we can put four 96-well plates on the scanner surface, this mean we can acquire around 384 images at a time. When the scanner is restricted to capture specific region not all the plates, it works faster.
- Array of cameras: in this method, we can join 8 cameras as an array and stabilize them on a long holder and tool it up with a light source that will be put on the top or it can be at the bottom of the plate holder. The image capturing structure can use 6, 12, or 24 –well plate according to the number of cameras will be used. However, it will take more time to get the same number of images that will be taken by the scanner method. But, the resolution here will be higher than the scanner because the cameras have more focusing here regarding the less number of wells and the resolution of cameras is greater than the scanner.

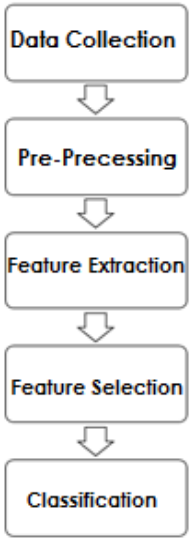


Figure 4. Stages of system methodology

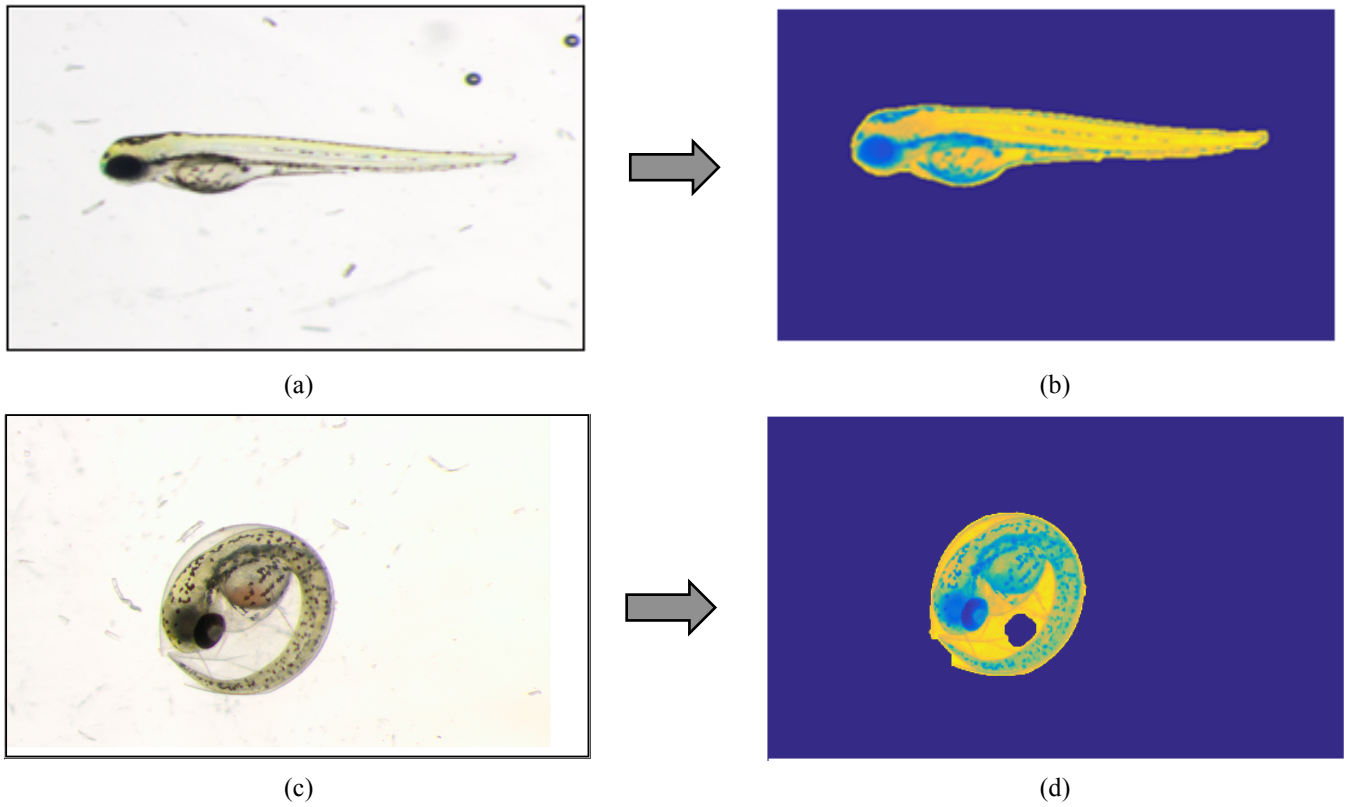
2) *Image pre-processing*: Numerous image-processing techniques can be used to enhance and modify the images. Having the most important information of the images will improve the classifier performance because it reduces any noise or distortion that may affect the desired detection. To generate modified images we applied several image processing functions to distinguish the larva body from the whole image, as shown in Fig. 5.

The resultant image is obtained from thresholding and labeling processes, in which the thresholded mask was found, using filtering and thresholding. Then the dilation and the erosion processes was applied to process the border of the object (larva) to be more obvious and recognized. As illustrated in Fig. 5, the raw image has many small objects around the larva body that should be eliminated to have clear features of the image using filtering and thresholding and another morphological operators, which yield the segmented image without any distortion.

3) Feature extraction: It is the process in which the most important information of the image is taken and the useless data is discarded. These features are used in machine learning by comparing its values for the trained dataset to classify them according to these features. Having more features in the images increase the performance of the recognition process in which the similarity between images may happen. In the proposed system there are three types of the features related to the shape, texture, and the intensity in the image. The shape includes the circularity, the area, and the perimeter. The intensity is calculated by the mean, median intensity, and standard variance. Likewise, the texture features are presented by contrast, homogeneity, and correlation.

4) Feature selection: This step aim to reduce the redundancy of the features. To avoid finding the features more than once the most important features will be selected. The selection process depends on the nature of the classes and the required information from the images. In the proposed system we used the shape features to classify two classes (normal and chorion).

5) Classification: The collected image dataset is divided into two groups; one for training (60%) and the other for test (30%) and validation (10%). This stage aims at identifying whether the life status of the embryos under test (i.e. alive or dead) as well as the shape of its growth (i.e. normal or abnormal). The pre-processed images are going into the ANNs multi-class classifier with the selected features to learn the system which patterns should be recognized. Finally, give the user a status report about the collected images of the larvae. The proposed system starts with two classes that are the chorion (non-hatched) and the normal shape of embryo. This will be extended for the other types of malformations. To classify the normal and the chorion types we need the shape features that is presented by the circularity shape of the embryo body.



(a) (b)

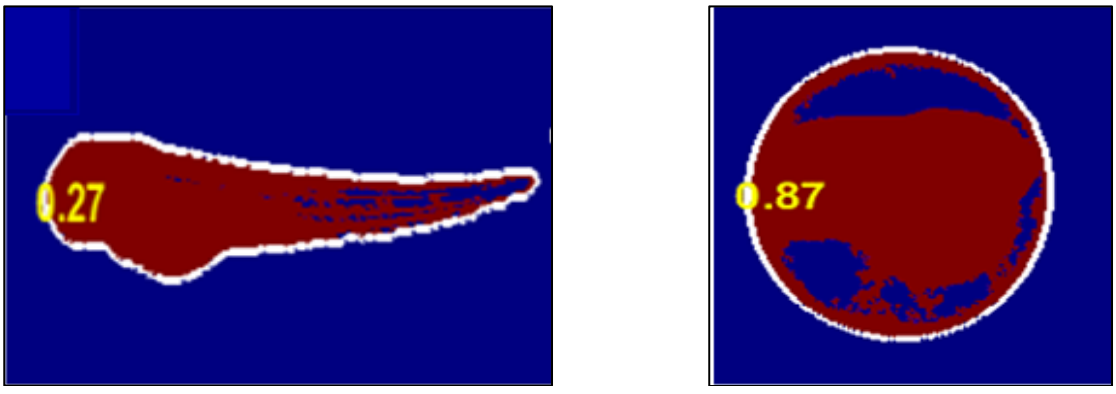
(c) (d)

Figure 5. Examples of abnormal embryo images; before processing (a, c) and after processing (b, d)

IV. Results and Discussion

The precursory results of the proposed system showed a good results in capturing process using scanner because it gives the images in a fast manner and a good resolution. The second way for image capturing will be tested in a future work. The proposed approach efficient NN model that could detect one type from the malformation types which is chorion. A shape-comparison between a normal and chorion larva is shown in Fig. 6.

Performance of the developed ANN classifier is demonstrated in the confusion matrix of Fig. 7, which is 97.7%. It can be noticed that this classifier has successfully distinguished 173 images out of 177 images for both classes. In this matrix, class 1 presents the chorion phenotype where class 2 presents the normal shape of the embryo. The number of recognized images in the present classifier will be extended to consider other types malformations.



(a) Normal (b) Chorion

Figure 6. Circularity of segmented larval outlines

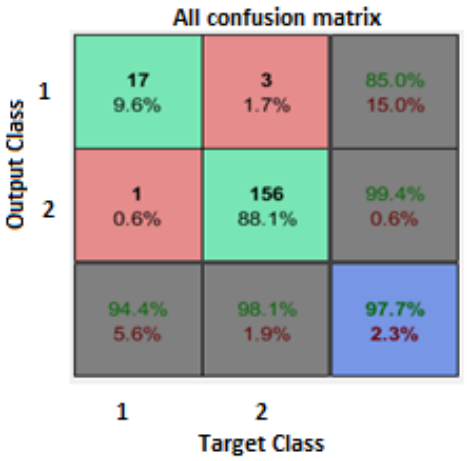


Figure 7. Confusion Matrix of the neural network Classifier

V. CONCLUSIONS

In this paper, a high-throughput automated approach for detection and classification the zebrafish larvae malformation has been presented and discussed. This proposed approach present a high-throughput screening to detect the malformation of the zebrafish larvae automatically that will mainly reduce the consuming time with manual data analysis that should be done by an expert biologist and also getting information about the experimented doses in a short time with high accuracy and low cost. The automatic and parallel capturing process will rise the system speed while taking group of images rather than the individual and manual process. Using a high performance classification system will recognise and detect the malformation in fast, accurate and precise way. The automated system with zebrafish larvae has been done via tracking or specific part of the body detecting, where the malformation detection system still need a new algorithms to be high-throughput and more accurate regarding to the system demands. The preliminary results showed that using NN as a classifier model give a high performance classification with a low recognition error in detecting the embryos malformation. This work will be applied to other types of the malformation after extracting another features from the images which is ongoing work.

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