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High-throughput Screening Platform for Quantitative Phenotype Analysis of *Xenopus laevis* with Deep Learning

[1]Hyunmo Yang, [1]Sanzhar Askaruly, [1]Seongmin Yun, [1]Geoseong Na, [1]Taejoon Kwon, and [1]Woonggyu Jung*
[1]Department of Biomedical Engineering, Ulsan National Institute of Science and Technology, Korea

ABSTRACT

Xenopus laevis are emerging models to study human diseases and to investigate pharmaceutical effects *in vivo* due to smaller size and faster developmental rates. It is also an effective organism to observe drug effects on phenotypic characteristics because it can provide many biological systems in a short time and remain optically accessible at the early stages of development. Although morphological evaluation of massive *Xenopus* data is an essential procedure, it requires labor-intensive and manual inspection under an optical microscope. In this study, we propose a high-throughput, wide-field, and time-lapse phenotype screening system modifying the office scanner. We also fabricated the customized PDMS well plate for efficient and stress-free imaging of living *Xenopus laevis* samples in normal and drug environments. With our manipulated device, we were successfully able to monitor the morphological changes of *Xenopus laevis* embryos acquired from more than 180 wells throughout 72 hours post fertilization stage. Our home-built software combines best practices of image processing and deep learning for automated accurate segmentation of large *Xenopus* data. Importantly, phenotypic features are quantitatively extracted to monitor the early-stage morphological abnormalities. In addition, the convolutional neural network (CNN) based algorithm enable to classify phenotype precisely. In conclusion, compared to conventional microscope screening, our platform offers high-throughput, accurate, and fast quantitative phenotype analysis. The suggested platform could become a promising tool in massive and dynamic observation such as developmental studies, drug testing, and phenotype-genotype assays, where statistical knowledge is critical.
Keywords: high-throughput, drug screening, phenotype screening, developmental studies, quantitative image analysis, scanning platform, deep learning

1. INTRODUCTION

Accurate and standardized measurement of high-dimensional phenotypic data, called phenomics, is essential to expand our knowledge for gene and environment effects on phenotypic changes [1]. The conventional microscope has been served as primary equipment for the phenotypic screens for early embryo development in various model organisms, but the microscopic equipment for high-throughput screening is quite expensive. Alternatively, the flatbed scanner has been considered to obtain quantitative images for large-scale phenotype assay. Compared to the conventional microscope, the large field-of-view (600 ~ 700 cm2) and low cost are suitable to use this equipment for large-scale phenotype assay [2]. Here we present the flatbed-based phenotype assay platform to analyse the phenome of the African clawed frog Xenopus laevis. Utilizing the power of deep learning approach with massive amount of data, we have confirmed the potential of suggested platform for the quantitative phenotype analysis along the developmental and the environmental changes. Moreover, the high-throughput screening capability achieved based on the convolution neural network classifier.

2. METHODS

2.1 System setup

We suggest an integrated platform combined of a modified commercial scanner (HP ScanJet G3110) and a custom microplate to obtain enhanced, artifact free embryo images. The cover part of scanner is equipped with automatically controlled LED device of white light (6,500K) to neglect background shadow effect. Moreover, we aim to capture the maximal contrast between naturally yellow tone *Xenopus laevis* and background with blue cellophane as shown in Figure 1 (A1) and (A3). In order to resolve edge and reflection artifact, present during imaging with 96-well plate, we also developed a customized 61-well (120mm x 85mm) PDMS microplate for high-throughput screening. Additional gradient for rectangular wells is introduced to bypass the forming edge artifact, while attached circular polarizer aids to prevent arising reflections (Figure 1, A2).
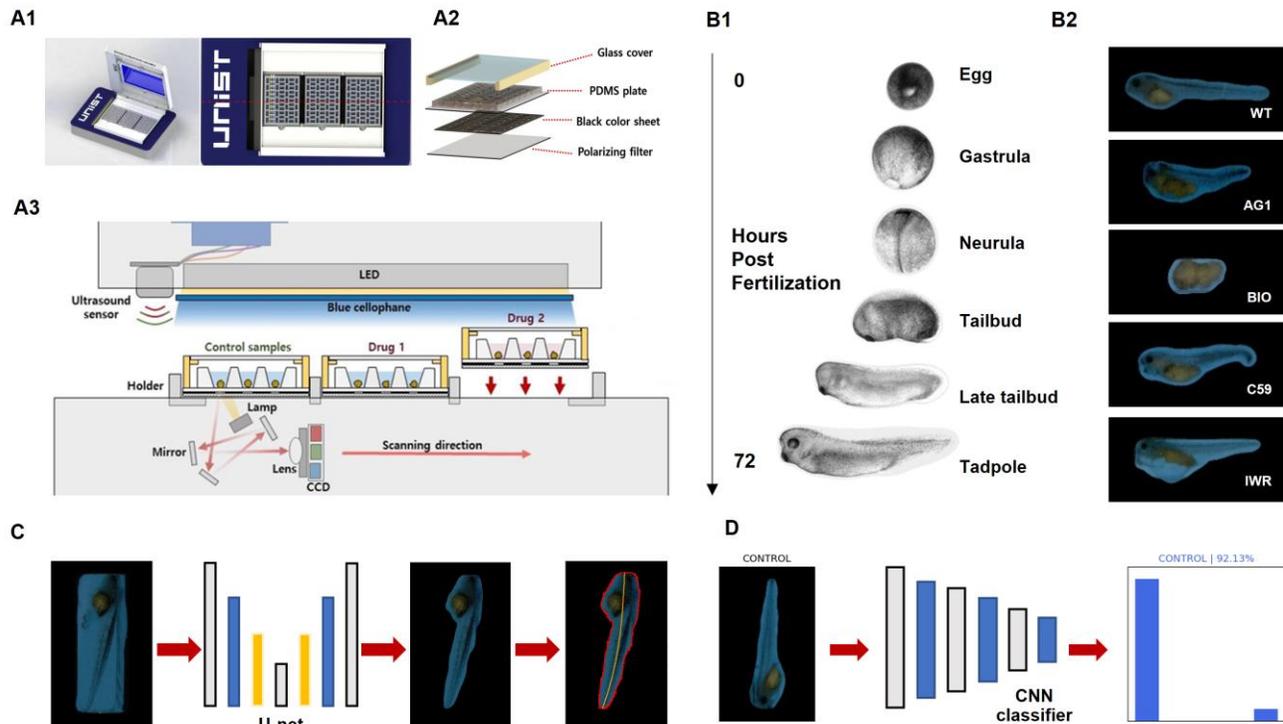
Figure 1. Overview of proposed platform. (A) Modified flatbed scanning system and designed PDMS microplates; (A1) Scanner, (A2) PDMS microplates, (A3) Cross section view of modified scanning system, (B) *Xenopus laevis*; (B1) Lifecycle for 72 hours post fertilization, (B2) Representative phenotype expressions induced by each drug type, (C) Quantification procedure of morphological parameters; for segmentation we trained and utilized U-Net, (D) Classification example using the CNN classifier.

2.2 Animals and drug treatment

The *Xenopus laevis* embryos were obtained using standard protocols as described [3]. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Ulsan National Institute of Science and Technology (UNISTACUC-16-14). Adult *laevis* females were briefly injected with human chorionic gonadotropin and stored for a day at the incubator to induce ovulation. Afterwards, we squeezed the frog gently to lay the eggs and performed in vitro fertilization. When the embryos reached the 4-cell developmental stage, the jelly layer of fertilized eggs was removed with 3% L-cysteine solution based on 1/3X MMR. Embryos with regular cleavage shapes were then manually picked and deposited to each well of the plate by pipette.

For the observation of phenotypic changes, the following drug treatment was applied. We used BIO (GSK-3 Inhibitor IX; B1686) and AG1 (BML-284) to activate the WNT signaling pathway during development. Similarly, we utilized IWR (IWR-1-endo) and C59 (Wnt-C59) to inhibit the WNT pathway. We dissolved each drug in DMSO and treated them at post-fertilization with specific concentrations in the media correspondingly. Figure 1 (B1) and (B2) shows representative phenotypic changes along the early lifecycle and the final stage phenotype induced by each drug.

3. RESULTS

3.1 Quantification of morphological changes

In this study, we present quantitative evaluation of embryo's phenotypic changes from morphological perspective. Using deep learning technique for segmentation, we acquired statistically powerful number of corresponding masks for further comprehensive analysis (202,474 samples). We investigated four drug effects on geometrical parameters: area, perimeter, length, and circularity as shown in Figure 2. Our results contribute to observation of variant development dynamics as well as assist early differentiation signals. Specifically, tracking the beginning of hatching stage (~30 hpf), or alterations in growth of tail (>60 hpf) could provide meaningful insights for discovery of drugs, designed to target certain pathways.
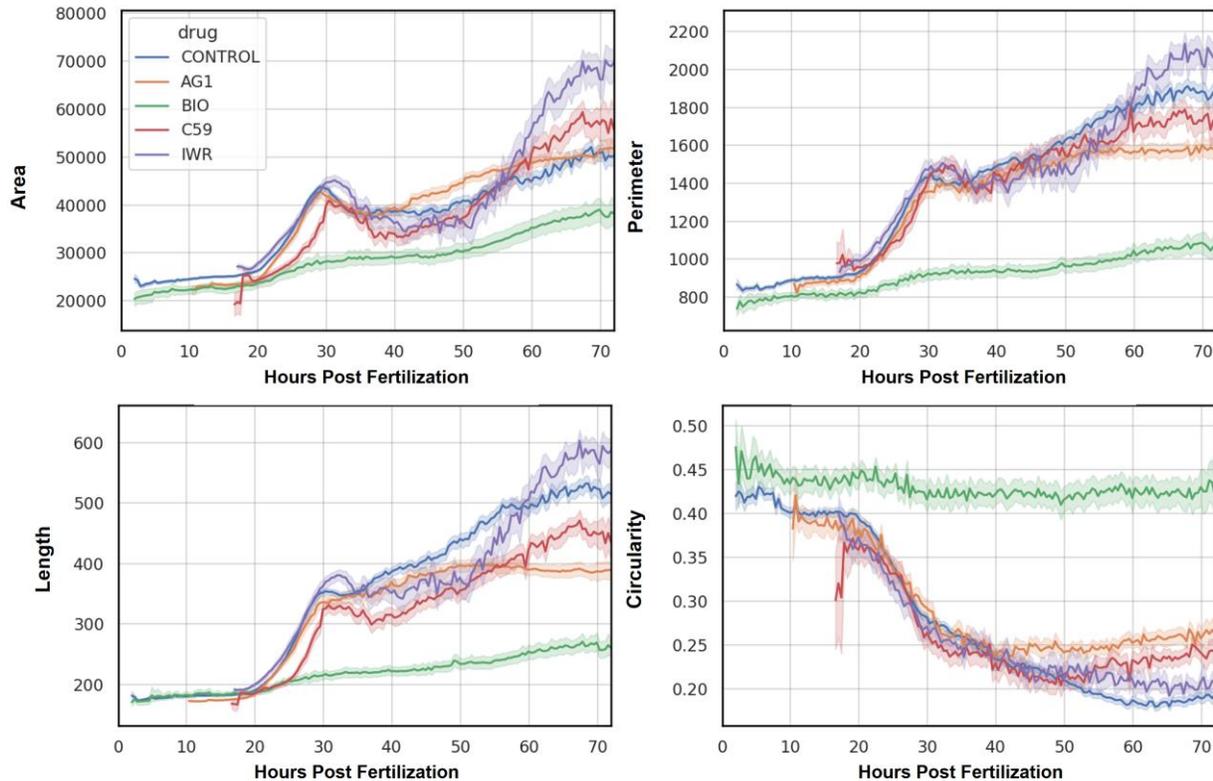

Figure 2. Quantification of morphological changes in terms of area, perimeter, length, and circularity. Area, length, and perimeter measured in number of pixels.

3.2 Classification of drug treated samples with convolutional neural network for high-throughput screening

Although the massive analysis on morphological changes of different phenotypic expressions, that induced by drugs, provide quantitative variations along the observation time, it is not sufficient to differentiate clearly that which type of drug were treated before dramatic morphological changes happened. For the high-throughput screening in real-time, therefore, we further developed the classification model based on CNN which extract 2-dimentional information from scanned embryo images. We trained the CNN classifiers for following developmental stages of Xenopus laevis in which early egg (~20.4 hpf), hatching (20.4 ~ 27.9hpf), tail development (27.9 ~ 55hpf), and grown after tail (55hpf ~) to confirm the screening functionality at each typical developmental period of Xenopus laevis as shown in Figure 3 (A).

Figure 3 (B) shows classification results as heatmap of predicted labels compare to true labels from the validation dataset. In the egg and hatching stages classification accuracy is not sufficient for all drug types, however, along the tail development stage, BIO, C59, and IWR treated samples were classified with 90%, 86%, and 79% accuracy while the classification accuracy of AG1 treated and CONTROL (CTL) samples were over 70% in the grown after tail stage. The summary of classification is shown in Figure 3 (C) as the confusion matrices for each development stage.
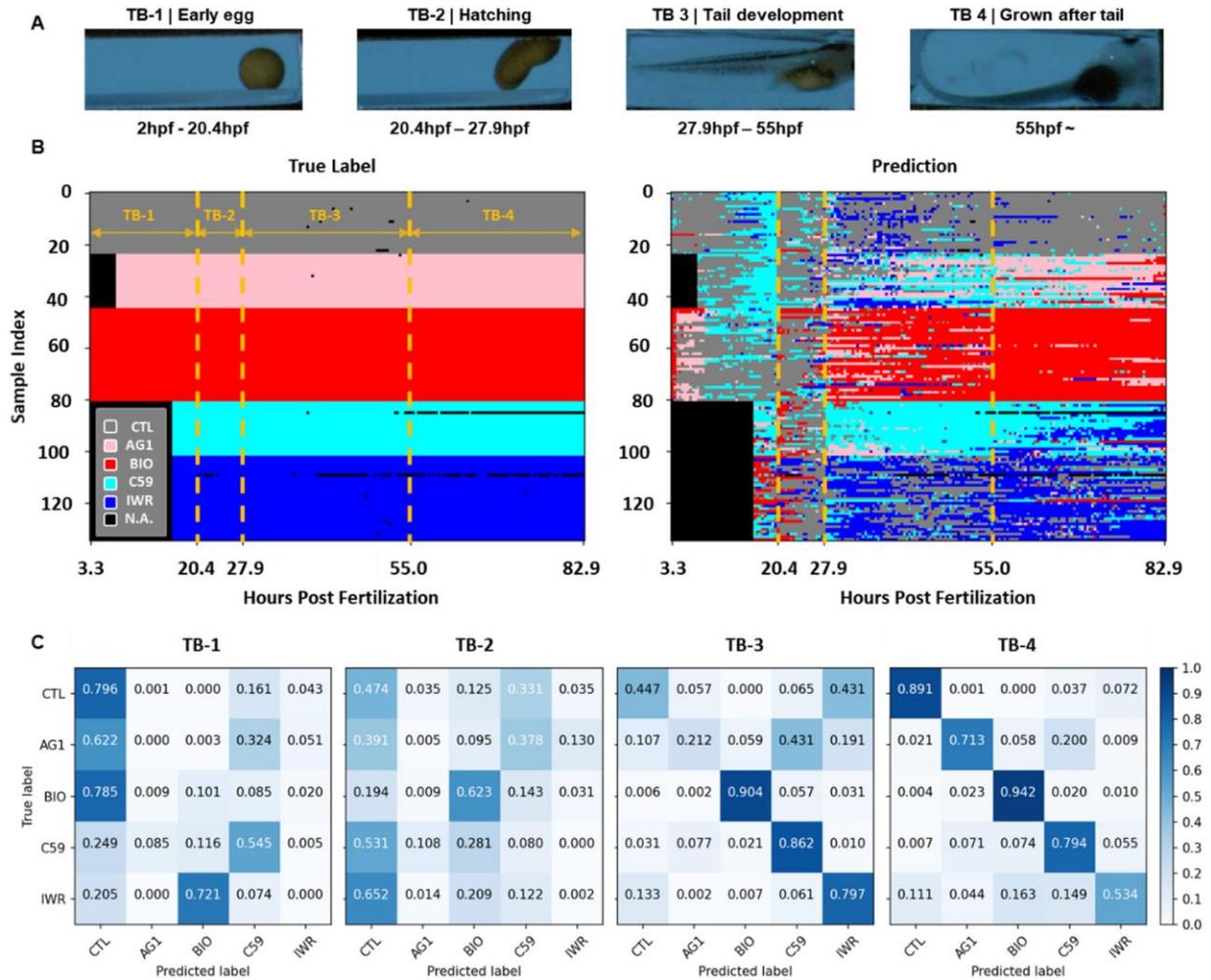
Figure 3. Classification results along developmental stages. (A) Representative phenotypes of healthy sample along the divided stages (TB: time block). (B) Heatmaps of true and predicted labels from validation dataset (N=135). (C) Confusion matrices of classification results for each stage.

4. CONCLUSION

In this study, we developed the high-throughput screening platform with modifying flatbed office scanner and utilizing deep learning technique. Based on the power of massive data acquisition and the deep learning based image processing, our platform offers high-throughput, accurate, and fast quantitative phenotype analysis, and the screening capability. The proposed platform could become a promising tool in massive and dynamic observation based biological studies, such as developmental studies, drug testing, and phenotype-genotype assays.

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