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Invited review
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Computational techniques in zebrafish image processing and analysis

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HIGHLIGHTS

- We reviewed the recent development in zebrafish microscopic image processing techniques.
- Zebrafish is a widely used vertebrate animal model in many studies.
- Microscopic images of zebrafish have many unique features, thus requiring judiciously development of dedicated algorithm for analysis and quantification.

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ABSTRACT

The zebrafish (Danio rerio) has been widely used as a vertebrate animal model in neurobiological. The zebrafish has several unique advantages that make it well suited for live microscopic imaging, including its fast development, large transparent embryos that develop outside the mother, and the availability of a large selection of mutant strains. As the genome of zebrafish has been fully sequenced it is comparatively easier to carry out large scale forward genetic screening in zebrafish to investigate relevant human diseases, from neurological disorders like epilepsy, Alzheimer's disease, and Parkinson's disease to other conditions, such as polycystic kidney disease and cancer. All of these factors contribute to an increasing number of microscopic images of zebrafish that require advanced image processing methods to objectively, quantitatively, and quickly analyze the image dataset. In this review, we discuss the development of image analysis and quantification techniques as applied to zebrafish images, with the emphasis on phenotype evaluation, neuronal structure quantification, vascular structure reconstruction, and behavioral monitoring. Zebrafish image analysis is continually developing, and new types of images generated from a wide variety of biological experiments provide the dataset and foundation for the future development of image processing algorithms.

Contents

1. Introduction

As a vertebrate animal model, the zebrafish has attracted much research interest because its embryos are transparent, allowing them to be imaged by optical microscopes (Kari et al., 2007) for various studies such as those addressing neurodegenerative conditions like Alzheimer's and Parkinson's disease (Bretaud et al., 2007; Newman et al., 2009; Xia, 2010). The objectives of the imaging experiments of zebrafish range from investigating its phenotypes in response to environmental cues and drug administration and development of its neuronal structures to elucidating its blood vessel formation at different development stages as well as tracking the dynamic behavior of live zebrafish under microscopes. Phenotypic screening using zebrafish is fast and cost-effective as compared

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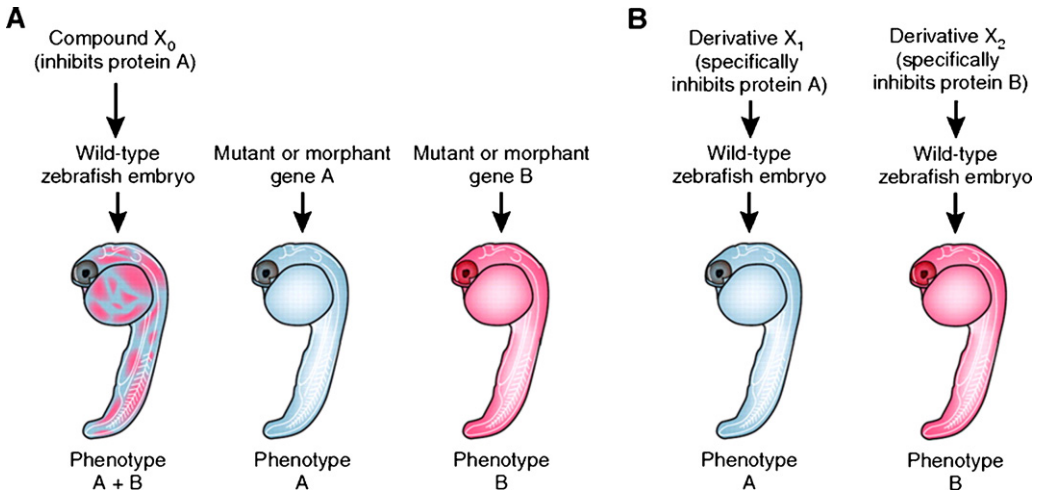

Fig. 1. The use of zebrafish embryos to assess off-target effects of drugs. Zebrafish mutants provide a blueprint for the effects of the loss of activity of proteins and, in combination with compound derivatization, can aid the development of drugs with greater specificity in whole animals. (A) Wild-type embryos treated with compound X_0, designed as an inhibitor of protein A, show phenotype B in addition to the phenotype A that is seen in embryos mutant for protein A. Phenotypic data of zebrafish mutants can be used to determine that the nature of the off-target effect is similar to that caused by a mutation in gene B. Therefore, the results of the assay indicate that the encoded protein B might be another target of the compound X_0. (B) Derivatization of compound X_0 yields specific compounds X_1, which induce a phenotype in zebrafish embryos resembling knockout of gene A only and X_2, which induces a phenotype resembling knockout of gene B only. This process in an intact animal can lead to the elimination of the drug's off-target effects by derivatization of compound X_0.

Used with permission from Strahle and Grabher (2010).

with other animal models like mouse. Thus zebrafish is becoming one of the most popular species in high-throughput screening. Because its embryos are transparent, its neuronal structures and blood vessels can be directly observed under a microscope, providing researchers an opportunity to characterize aberrant neuronal and vascular patterns that are due to gene manipulation or therapeutic interventions. As a result zebrafish has been proven to be a valuable model for studying many neurological and cardiovascular diseases and widely used in our search for new therapies for neurodegenerative and heart diseases. Another important application of zebrafish is in neurobehavioral study because zebrafish has robust neuroendocrine responses that are closely correlated with its behaviors such as its response to stress, predators, alarms, and drugs. Hence, zebrafish are quickly becoming a popular model in behavioral studies wherein its swimming pattern and schooling behavior are recorded by video equipment for analysis. In most of the above applications, the zebrafish-based experiments generate image data in such a large number that it is often difficult to analyze by a manual process. As most of the zebrafish imaging experiments generate image data in such a large number that it is often difficult to analyze manually. Correspondingly, there is a growing requirement for computational techniques to process, analyze, and quantify zebrafish images. Meeting such objectives around the managing and processing of zebrafish image data is important to enable it to reach its potential in promoting biological discovery and understanding.

Compared with other animal models, the zebrafish has other characteristics that make it well-suited for imaging-based experiments. For example, the species has a relatively high reproduction rate and a short hatching period (less than 72 h post fertilization (hpf)); therefore, they are easy to breed and immediately available for experimental manipulation. The large number of embryos from each breeding pair of zebrafish allows researchers to test various conditions in a high-throughput manner (Letamendia et al., 2012). The small sizes of embryos allow them to be assayed in microtiter plates for quick image acquisition. The above advantages make the zebrafish well-suited for both gain-of-function and loss-of-function studies in neurobiology (Hogan et al., 2008; Key and Devine, 2003). *In vitro* transcribed mRNA and plasmid DNA are commonly used to produce transient expressions in the zebrafish for gain-of-function investigations (Adam et al., 2000; Hjorth et al., 2002; Scheer and Campos-Ortega, 1999). Using anti-sense technology such as morpholinos to knockdown gene expression (Nasevicius and Ekker, 2000) and dominant negative techniques (Anderson et al., 2000) to block endogenous protein interactions, researchers have also used the zebrafish for loss-of-function studies.

In addition to transparent embryos, a high reproduction rate, and a short hatching period, zebrafish has another reason that is driving their wide applicability in research and, that is, the relative ease of engineering zebrafish mutants for whole body microscopic imaging. Embryos of zebrafish mutants have been utilized in rapid whole-animal drug-specificity assessments (Strahle and Grabher, 2010). For example, one can use zebrafish mutants to assess the effects of the loss of activity of a protein in the presence of a specific drug that inhibits the expression of the protein. As Strahle and Grabher show in Fig. 1(A), wild-type embryos treated with a compound that inhibits a protein show phenotype B, while phenotype A is caused by the mutation of the protein. In addition, one can specifically test the off-target effects of a drug that is designed to induce or inhibit a phenotype in zebrafish embryos by knocking out certain genes. As shown in Fig. 1(B), one can characterize the phenotypes induced by two compounds that are designed to knock out different genes and compare the phenotypes in the intact animals for the compound's off-target effects (Strahle and Grabher, 2010).

The above advantages make the zebrafish widely applicable in diverse biological experiments, particularly those with imaging as a primary investigative tool. In either a regular or high-throughput imaging setup, zebrafish-based experiments typically generate a large number of images in 2D, 3D, or even 4D when time-lapse imaging is applied. For example, Kaufamm et al. developed protocols that allow optimal mounting of zebrafish embryos over several days by keeping them in a fixed position while providing enough space for the sample to grow (Kaufmann et al., 2012). Fig. 2 shows an example of time-lapse imaging of the same zebrafish embryo from 24 hpf to 96 hpf (Kaufmann et al., 2012). As shown in Fig. 2, for up to three days, a series of images can be taken to record the growth of the embryos and the development of vasculature.