Methods xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Tools for automating the imaging of zebrafish larvae

Rock Pulak

Union Biometrica, Inc., 84 October Hill Rd., Holliston, MA 01746, USA

ARTICLE INFO

Article history:
Received 5 October 2015
Received in revised form 24 November 2015
Accepted 25 November 2015
Available online xxxx

Keywords:
High-throughput
Image-collection
Automation
Zebrafish larvae
Drug screening

ABSTRACT

The VAST BioImager system is a set of tools developed for zebrafish researchers who require the collection of images from a large number of 2–7 dpf zebrafish larvae. The VAST BioImager automates larval handling, positioning and orientation tasks. Color images at about 10 μm resolution are collected from the on-board camera of the system. If images of greater resolution and detail are required, this system is mounted on an upright microscope, such as a confocal or fluorescence microscope, to utilize their capabilities. The system loads a larvae, positions it in view of the camera, determines orientation using pattern recognition analysis, and then more precisely positions to user-defined orientation for optimal imaging of any desired tissue or organ system. Multiple images of the same larva can be collected. The specific part of each larva and the desired orientation and position is identified by the researcher and an experiment defining the settings and a series of steps can be saved and repeated for imaging of subsequent larvae. The system captures images, then ejects and loads another larva from either a bulk reservoir, a well of a 96 well plate using the LP Sampler, or individually targeted larvae from a Petri dish or other container using the VAST Pipettor. Alternative manual protocols for handling larvae for image collection are tedious and time consuming. The VAST BioImager automates these steps to allow for greater throughput of assays and screens requiring high-content image collection of zebrafish larvae such as might be used in drug discovery and toxicology studies.

1. Introduction

Zebrafish (*Danio rerio*) have become an important vertebrate model organism for biomedical research [1,2]. Zebrafish embryos develop along a path shared with other vertebrates. Structural, functional and genetic similarities have led various groups to construct human disease models that have been used in phenotypic screening approaches for drug discovery and toxicology testing [3,4]. Additional features that make zebrafish ideal for this type of research includes their small size, optical clarity, that embryonic development occurs outside the mother, and the availability of a collection of molecular tools allowing genetic changes that mimic human disease states to be made [5,6].

Microscopy is an important tool for zebrafish researchers. The fertilized embryo starts out at about 1.2 mm diameter and this small size necessitates visual magnification to observe the development and function of the different tissues and organ systems. Imaging – collecting pictures and videos – provides the means of capturing the data for further analysis and archiving of the results of experiments.

Many advances have been made in microscopy over the years that allow for incredibly detailed image collection, especially fluorescence imaging and confocal imaging. However, traditional manual protocols for handling larvae for image collection are tedious and time consuming [10]. To automate image collection in a high throughput setting, methods for handling zebrafish larvae needed to be developed. The concept for one approach was proposed by Yanik [7,8] and involved automating a fluidics system to sequentially load young larvae to a capillary tube for orienting and imaging. This approach has been developed into a collection of tools called the VAST BioImager. It automates the handing of 2–7 dpf larvae for high throughput and high content image collection. The larvae to be imaged may be live, anesthetized, or fixed. This report describes the VAST BioImager system and how it works to capture images from many young larvae in an automated and high throughput process (see Figs. 1–5).

2. Description of VAST BioImager

The VAST BioImager is composed of two separate pieces of hardware, along with a computer and monitor. One of these, the imaging unit, contains the capillary in which the larva is held during imaging and the on-board color camera for initial larvae identification and orientation. The second is a controller unit that

Abbreviations: dpf, days post fertilization; fps, frames per second; mL, milliliter; mm, millimeter.

E-mail address: rpulak@unionbio.com

http://dx.doi.org/10.1016/j.ymeth.2015.11.021
1046-2023/

Fig. 1. VAST BioImager is composed of two parts, the imaging unit (left) and the controller unit (center). A computer/monitor runs the software that controls the VAST BioImager and collects the larval images.

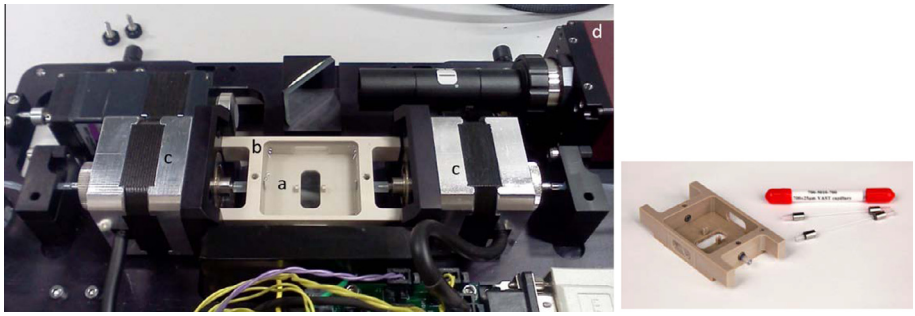

Fig. 2. (left) VAST BioImager imaging unit showing the location of the capillary (a) in the water tray (b), flanked by the rotation motors (c). The on-board camera (d) captures images of the capillary off of the mirror. (right) Image of water tray and replacement capillary assemblies.

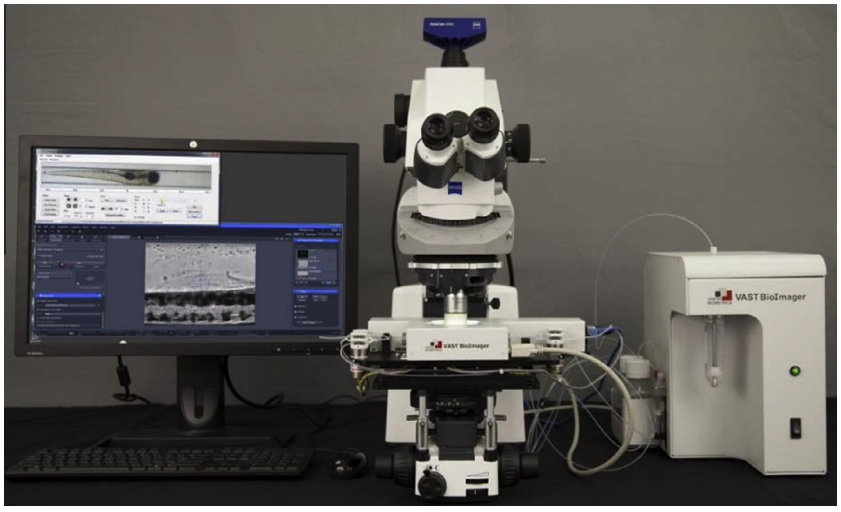

Fig. 3. VAST BioImager attached to the stage of an upright microscope (Zeiss Axio) for imaging at a higher resolution and capturing fluorescent images.

carries the electronics, the fluid syringe for pulling the larvae from the bulk reservoir and pushing them through to the capillary for imaging and fluid reservoirs used to accomplish this. Tubing and electronics connect the two units together, valves on the imaging unit open and close to control the flow of larvae and fluids through the system. A computer, monitor and software integrate the two units to achieve the automation of image collection. Several of the individual components are described in greater detail.

2.1. Fluid syringe

A fluid syringe is responsible for moving fluids and larvae through the system. It is located on the controller unit and is connected to the buffer reservoir and the imaging unit of the VAST BioImager system by tubing. It functions in response to instructions from a script (controlled by software) to draw fluid from either the buffer reservoir or the sample line to load or unload the capillary with a fish.

Please cite this article in press as: R. Pulak, Methods (2016), http://dx.doi.org/10.1016/j.ymeth.2015.11.021