

3D Tomographic Visualization of Zebrafish Larvae Using the VAST BioImager™

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VAST and VASTomography™ features

- Automated rotation and high content imaging of zebrafish larvae
- Ability to generate 3D optical projection tomography in minutes
- Data sets of volume, shape and position for selected region of interest
- 3D visualization with VolView/ParaView
- Data processing with MATLAB/Python

Introduction

Tomography, imaging by sectioning, is a powerful three-dimensional visualization approach that has wide applications in medical and scientific fields. Similar to computerized tomography (CT), the process of obtaining 3D density distribution within a human body from multiple x-ray projections[1], VASTomography has the ability to enable 3D zebrafish research. Zebrafish, with its small size and optical clarity at larval stage, allows easy microscopic evaluation of physiological changes and morphological phenotypes. With the VAST BioImager[2], a tool for automated zebrafish larvae positioning, a large numbers of zebrafish samples can be quickly and automatically oriented and imaged from multiple angles without manual manipulation[3][4]. Here we present a tomographic visualization method for zebrafish larvae made possible by the VAST BioImager and the VASTomography software. Positional, volumetric and shape information can now be readily collected for further investigation.

Methodology

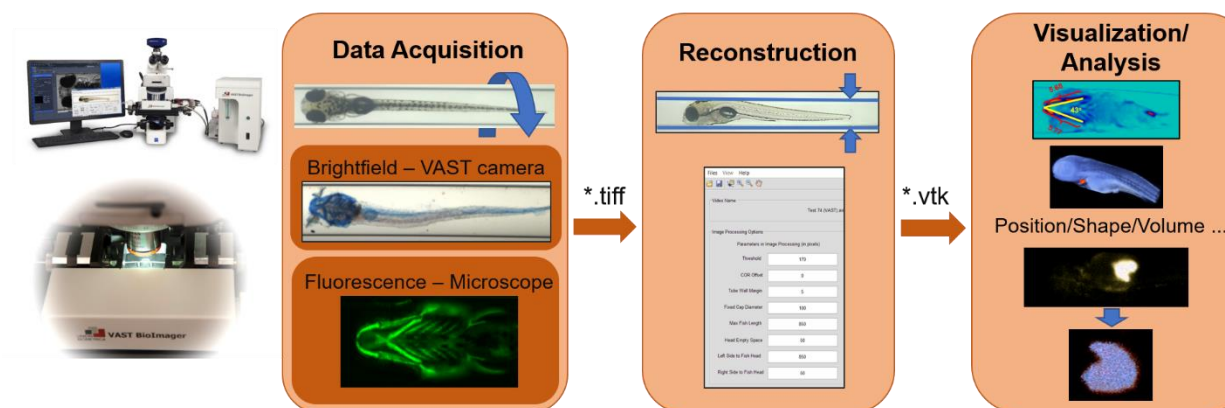


Figure 1. The workflow of generating 3D tomography using VAST BioImager and VASTomography software.

The VAST BioImager is mounted on a high-resolution upright compound microscope for automated positioning and imaging of zebrafish larva. A fish is loaded in a capillary and rotated through a full 360° rotation. Images are captured every 1 to 8 degrees. Such images include brightfield images generated from the VAST camera and fluorescent and brightfield images captured by the microscope. The collection time ranges from 3 to 33 minutes per larvae depending on the number of images captured. The image set is then fed to VASTomography software for optical projection tomographic reconstruction (OPT) which builds a 3D density model of the fish based on light transmission through the sample. The walls of the VAST capillary imaged by the VAST on-board camera are used as a guide for the center of rotation and optional software parameters allow for adjusting the area of focus for the reconstruction. A matrix file in VTK format is the output which can be analyzed in 3D visualization software such as VolView or ParaView. Thus, the user can now not only visualize the fish in 3D but also capture information such as position, shape and volume for further analysis.

Results

Here we report volumetric reconstructions of craniofacial features, hearts, and tumors in developing zebrafish using the VAST BioImager and the VASTomography software.

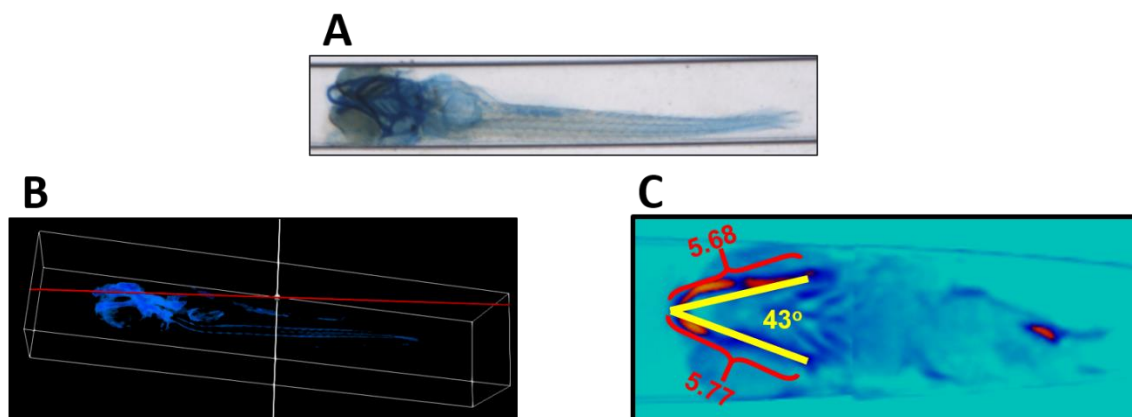


Figure 2. Constructing tomography from bright field images of 6 dpf zebrafish larvae stained with Alcian Blue. A) A brightfield image of a zebrafish larva by the VAST BioImager camera. B) 3D tomography of the same fish by VASTomography software. C) Craniofacial morphogenesis parameters such as jaw length and angle measured on a virtual slice along red plane in B. Imaged by VAST on-board camera.

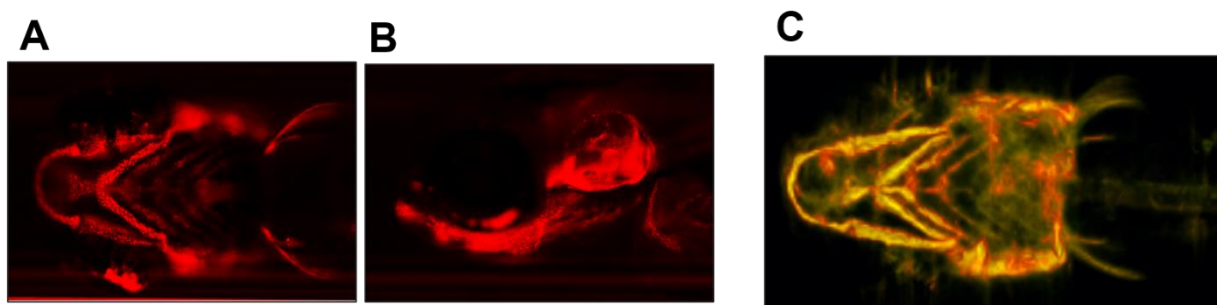


Figure 3. Constructing tomography from fluorescent images of 4 dpf zebrafish larvae expressing Col2-H2a-mCherry in craniofacial cartilage. A) Ventral view of the fish. B) Lateral view. C) 3D tomography of the same fish by VASTomography software. Imaged under a 2.5X 0.12NA objective (Zeiss).

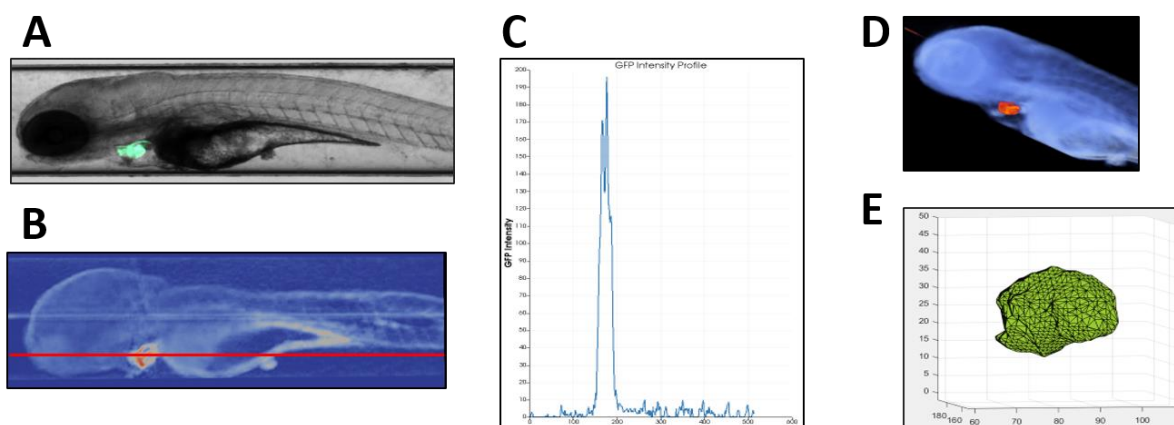


Figure 4. Measuring the heart volume in 8 dpf zebrafish larva where GFP is expressed in the heart epithelium. A) Image overlay of the brightfield and GFP channel of the 8 dpf zebrafish larva. B) a virtual slide of the 3D tomography. C) Pixel intensity profile along the red line in B. D) 3D tomography constructed by VASTomography software with the heart recognized and labeled. E) Volumetric measurement of the heart region recognized in D. Imaged under a 2.5X 0.12NA objective (Zeiss).

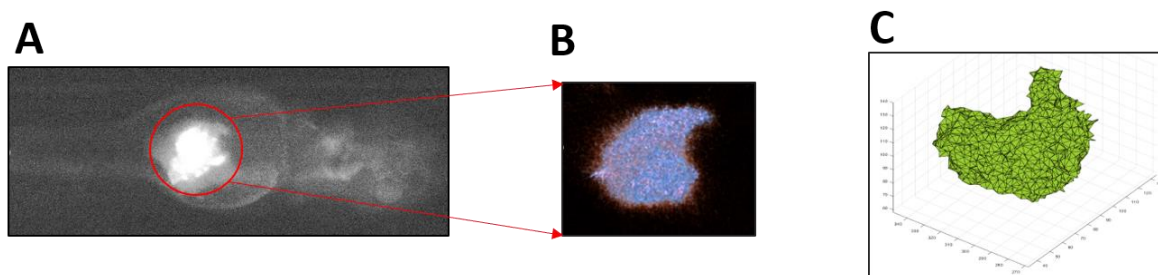


Figure 5. Measuring the tumor volume in 10 dpf zebrafish injected with human cancer cell line. A) Image showing the injected human cancer cells in the zebrafish. B) 3D tomography constructed by VASTomography software of the injected cells. C) Volumetric measurement of the injected cells. Imaged under a 2.5X 0.12NA objective (Zeiss).

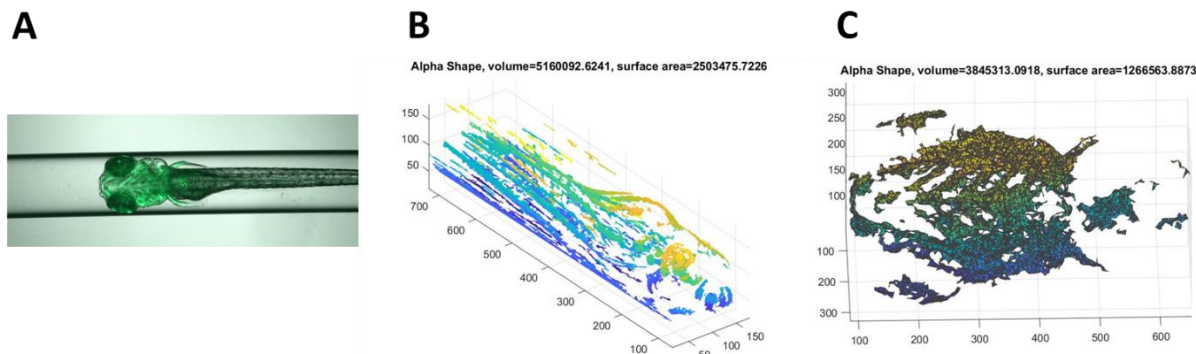


Figure 6. Measuring volume and surface area using the 'alpha shape' feature of the VASTomography software. A) An overlay image of the brightfield and GFP channels of a Fli-GFP zebrafish line (GFP reporter in the vasculature). Fish is 4-6 dpf and imaged under a 2.5X 0.12NA objective (Zeiss). B) Volumetric measurement (μm^3) and surface area (μm^2) of the whole fish, which is colored coded to better demonstrate a 3D view. C) Volumetric measurement and surface area of the head region of the fish.

Conclusions

VASTomography and the VAST BiImager can be used to generate 3D reconstructions of zebrafish larvae to achieve tomographic visualization and characterization in a high-throughput fashion. The 3D model enables the acquisition and analysis of morphological features at key stages of zebrafish embryonic development. The reconstructed tomography file can be visualized in open source software such as VolView or ParaView. The tomography matrix data can also be processed using MATLAB or Python. Notably the Alpha Shape feature of the VASTomography software generate real-time measurement of volume and surface area for regions of interests. Through the development of the method we found some key factors for successful data acquisition for 3D reconstruction: minimizing pigmentation in fish embryo, reducing embryo movement and using objectives with a field of view of 600 μm or more. Furthermore, the mechanical stability of the microscope and the embryo is essential when acquiring tomographic projection datasets.

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