3D Imaging of Zebrafish Larvae Using the VAST BioImager

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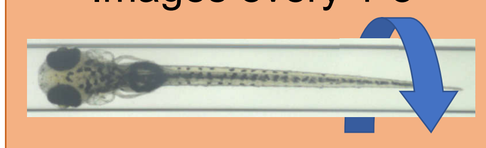
UNION BIOMETRICA
Large Particle Flow Cytometry

Methodology

VAST BioImager mounted on a high-resolution compound microscope for automated positioning and imaging of zebrafish embryos/larvae.

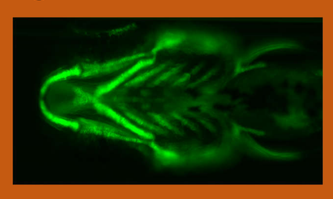
Data Acquisition
Full 360° rotation.
Images every 1-8°



Imaging capillary

Brightfield – images from VAST camera

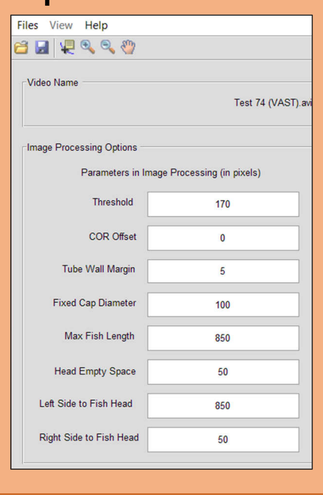
Fluorescence – FL+BF images from microscope



*.tiff

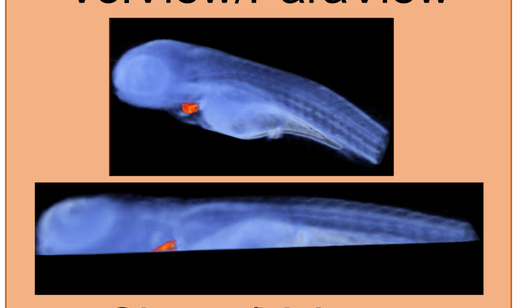
Reconstruction
Capillary wall - guide for center of rotation

Software to adjust reconstruction parameters

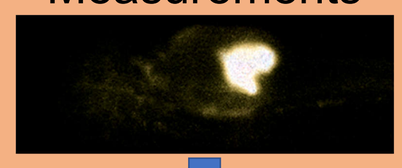


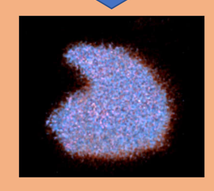
*.vtk

Visualization/ Analysis
VolView/ParaView



Shape/Volume Measurements





Introduction

3D tomographic visualizations have become a powerful approach in medical and scientific imaging. The mechanical stability of the microscope and the embryo is essential when acquiring tomographic projection datasets. Here we will report volumetric reconstructions of craniofacial features, hearts, and tumors in developing zebrafish using the VAST BioImager. 3D tomographic software enables the acquisition and analysis of morphological features at key stages of zebrafish embryonic development.

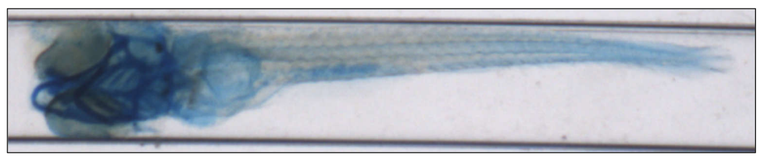
Results

Reconstructions were collected using a range of set angles from 0.72° per rotation to 7.92° per rotation, giving a range of collection times from 3 min to 33 min. This would impact the resolution of the reconstructions, however, the calculated volumes and surface areas using the same fixed larvae had no discernable difference from one another.

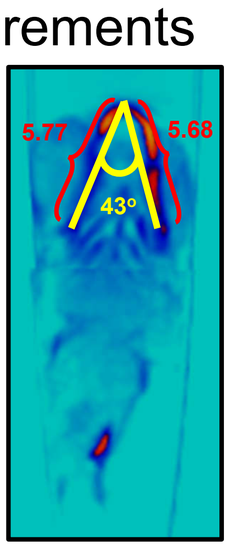
Potential applications of the approach:

1. **Craniofacial morphogenesis in development**

- **6dpf Alcian blue stained larvae**

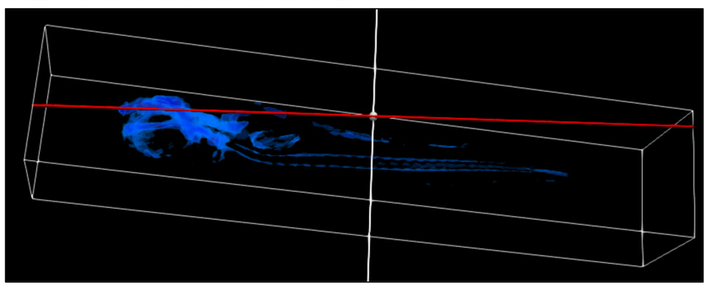


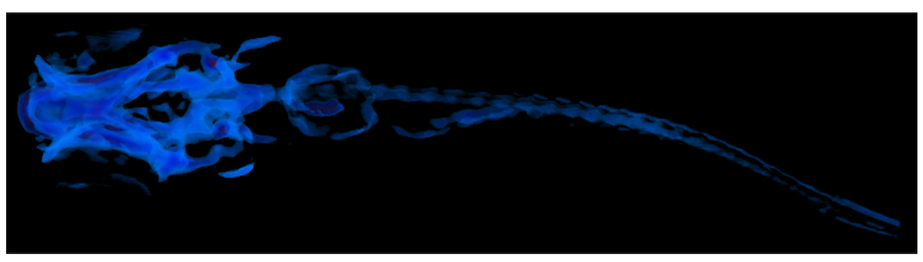
Length and angle measurements



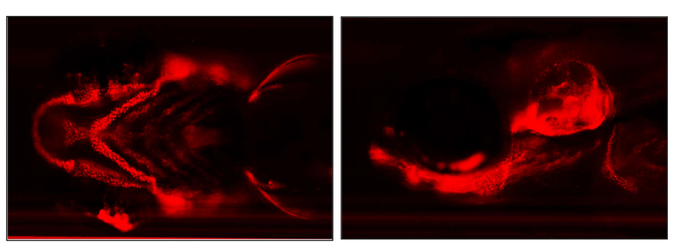
Virtual slice along red plane (line in this view)

3D reconstruction

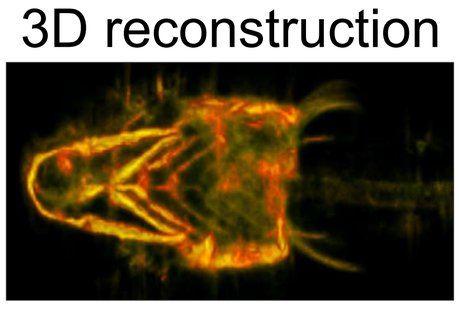




- **4dpf Col2-H2a-mCherry**

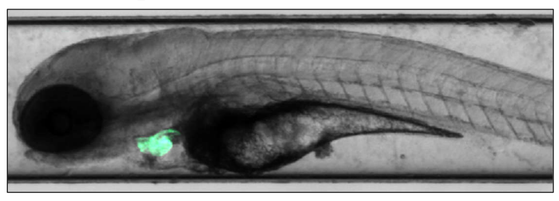


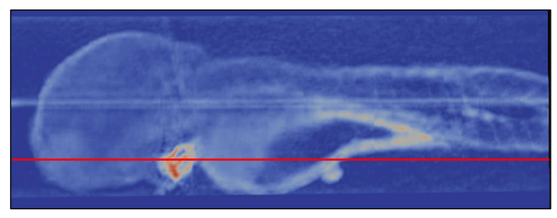
3D reconstruction



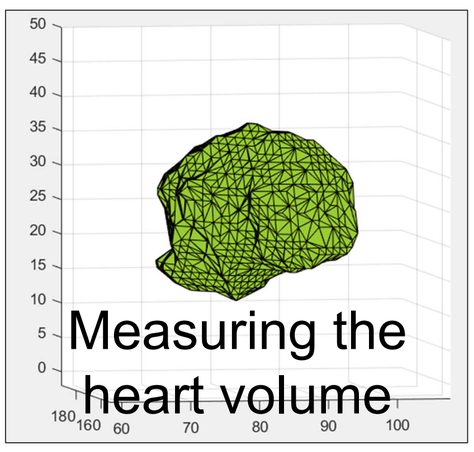
2. **Volumetric measurements in tissues**

- **8dpf, GFP in heart epithelium**

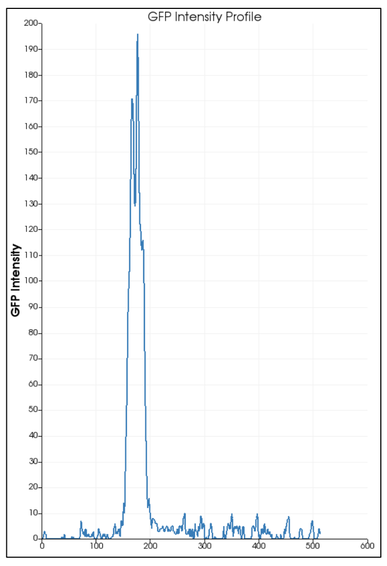


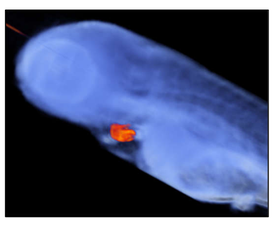


3D reconstruction

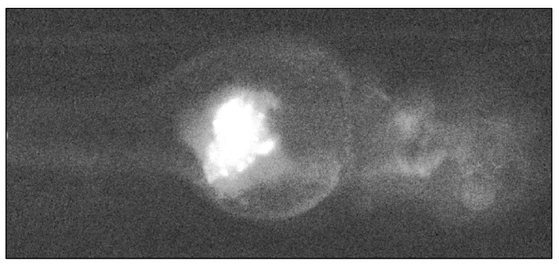


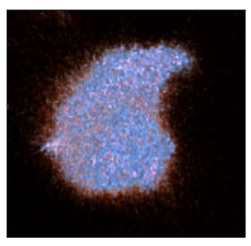
Intensity profile along red line



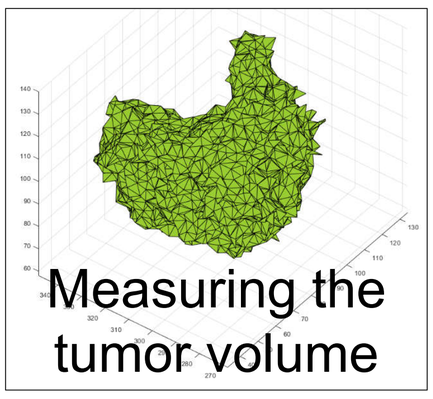


- **10dpf fish with human cancer cell line**





3D reconstruction



Conclusions

We developed a method and software for 3D reconstructions using the VAST BioImager. This methodology can be used for the high-throughput analysis of morphological features in zebrafish larvae. Key factors for successful data acquisition for 3D reconstruction:

1. Minimize pigmentation in fish embryo (PTU, KOH, etc.).
2. Reduce embryo movement (anesthetize or use fixed fish).
3. Prevent embryo from slipping in capillary (0.3% LMP Agarose).
4. Use objectives with a field of view of 600 µm or more.

We would like to thank Ela Knapik lab (Vanderbilt University), Stephen Byers lab (Georgetown University) and George Eisenhoffer lab (MD Anderson Cancer Center) for the help with test samples and transgenic zebrafish lines.