TRANSLATING DATA INTO DISCOVERIES

A MACHINE LEARNING-DRIVEN PHENOTYPING PLATFORM FOR RAPID *IN VIVO* TARGET VALIDATION AND PRECISION MEDICINE DEVELOPMENT

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TMLS CONFERENCE | JUNE 14 2023

MISSION

Translating Data into Discoveries

BioSymetrics is a phenomics-driven drug discovery company that generates data-driven insights from experimental and biomedical data, using machine learning, to translate complex human disease biology and advance **precision medicines**

Overview

Introduction

Data

Methodology

Results & Conclusions

Why Zebrafish? Powering translation with early *in vivo* models

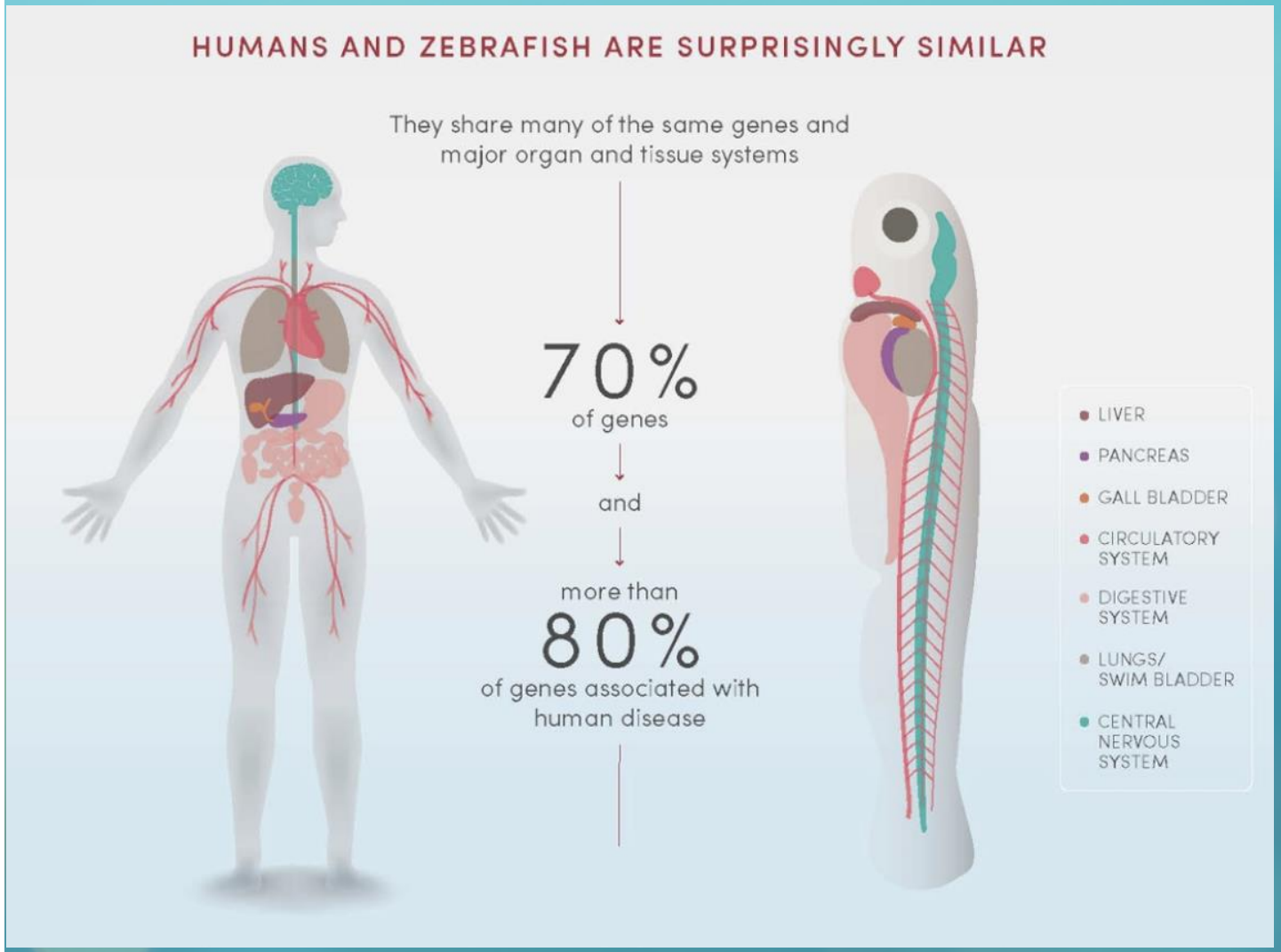
As our first model system, **ZEBRAFISH** offer

- Transparent, whole organism *in vivo* biology with beating hearts and developing brains
- Speed to target validation: 48 hours from CRISPR injection to observable phenotype
- Scalable screening in 96-well plates

Credit: Mirko Rosenau/Shutterstock.com

Why Zebrafish? Powering translation with early *in vivo* models

- 70% human genetic conservation
- Demonstrated translation for cardio and neuro programs where mouse models have failed[1]

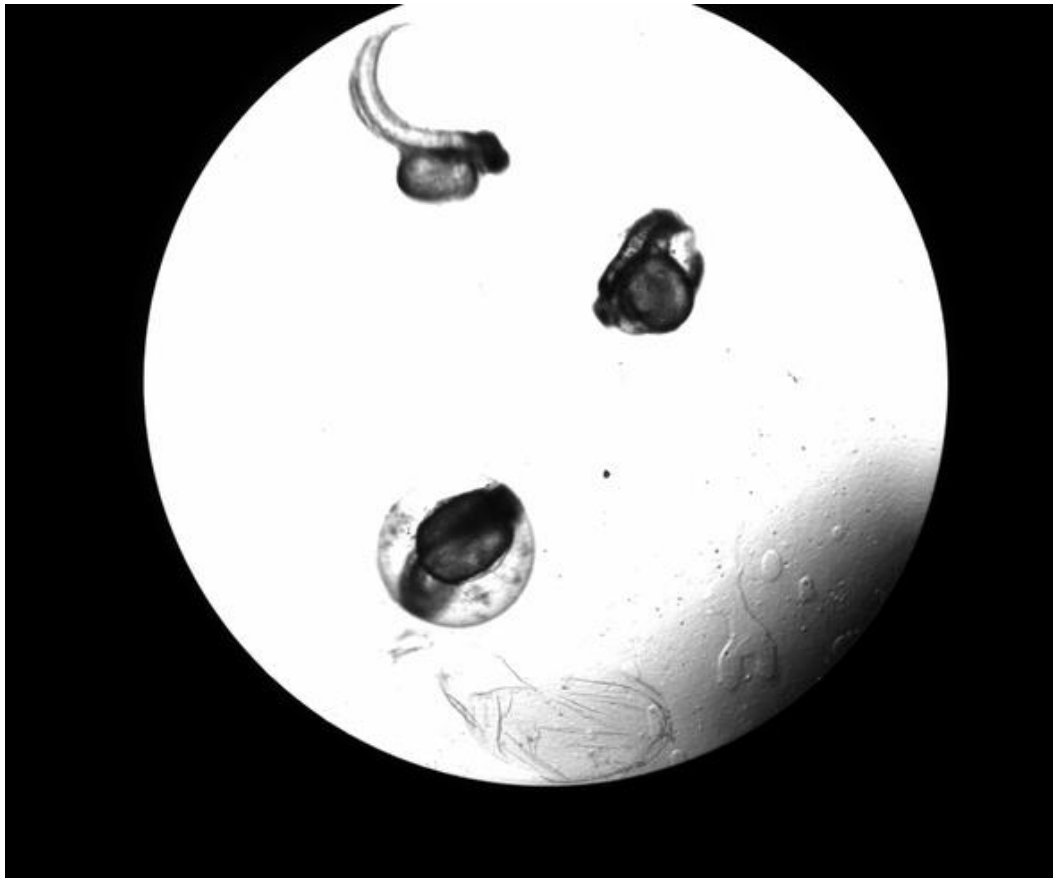


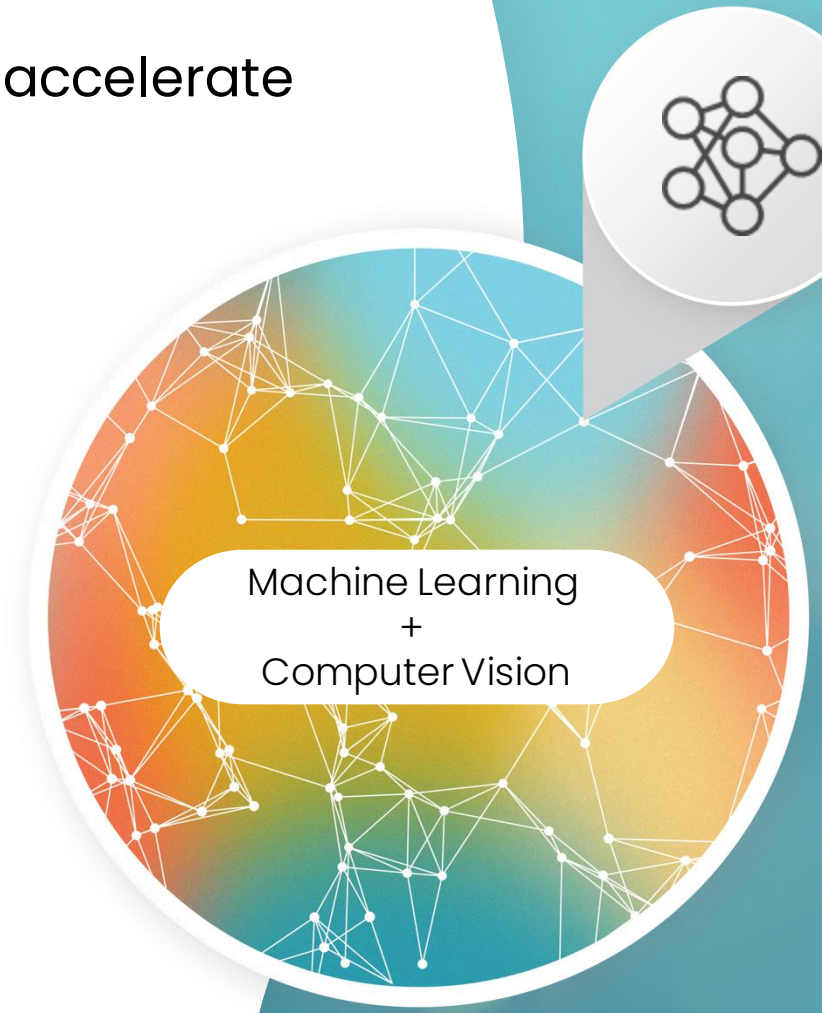
https://healthcare.utah.edu/healthfeed/postings/2017/11/zebrafish.php

[1] www.nature.com/articles/d41573-019-00165-x

Objective

Use machine learning and computer vision tools to accelerate **phenotype** analysis of zebrafish imaging data





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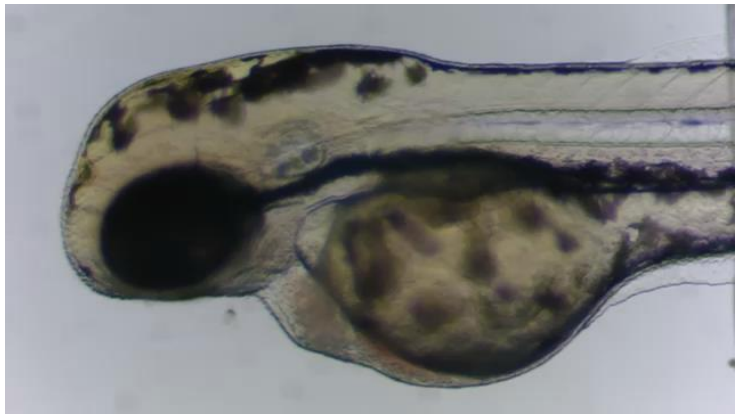
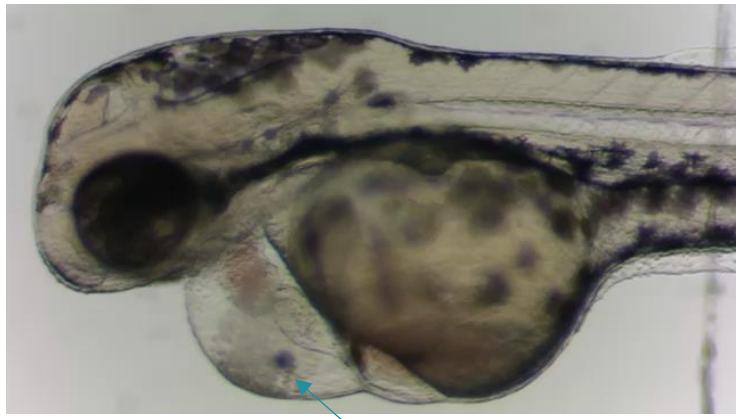
What is a phenotype?

A phenotype is an observable trait

Control



Mutant



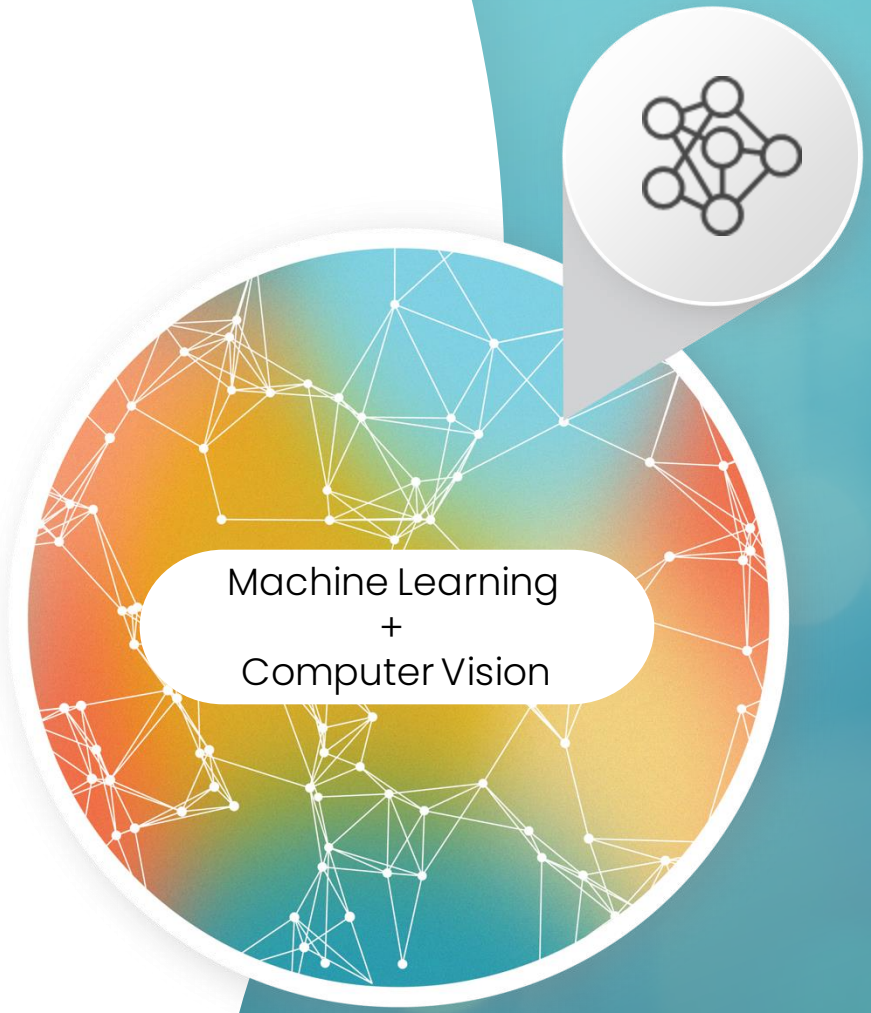
Enlarged heart region,
abnormal blood circulation

Other examples: hair colour, height, disease

A MACHINE LEARNING-DRIVEN PHENOTYPING PLATFORM FOR RAPID *IN VIVO* TARGET VALIDATION AND PRECISION MEDICINE DEVELOPMENT

We developed a zebrafish phenotypic screening platform that features:

- A custom 3D-printed mounting platform
- A fine-tuned deep learning instance segmentation model
- A computer vision post-analysis pipeline for quantitative analysis



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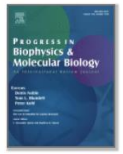
Working with Zebrafish

Humans and zebrafish exhibit similar ECG patterns, unlike the patterns seen in mice

ELSEVIER

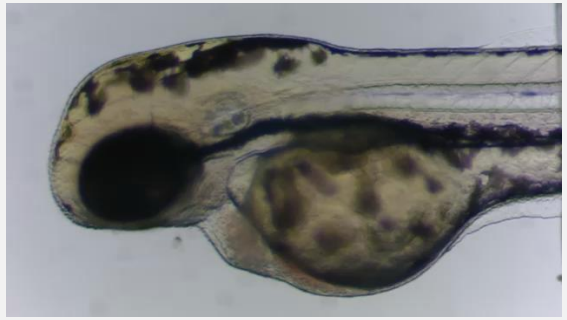
Progress in Biophysics and Molecular Biology

Volume 138, October 2018, Pages 132-141



Fish, the better model in human heart research? Zebrafish Heart aggregates as a 3D spontaneously cardiomyogenic *in vitro* model system

Patricia Hodgson [a, b], Jake Ireland [a, c], Bianka Grunow [d, a]



May 2018

DOI:10.1016/j.pbiomolbio.2018.04.009

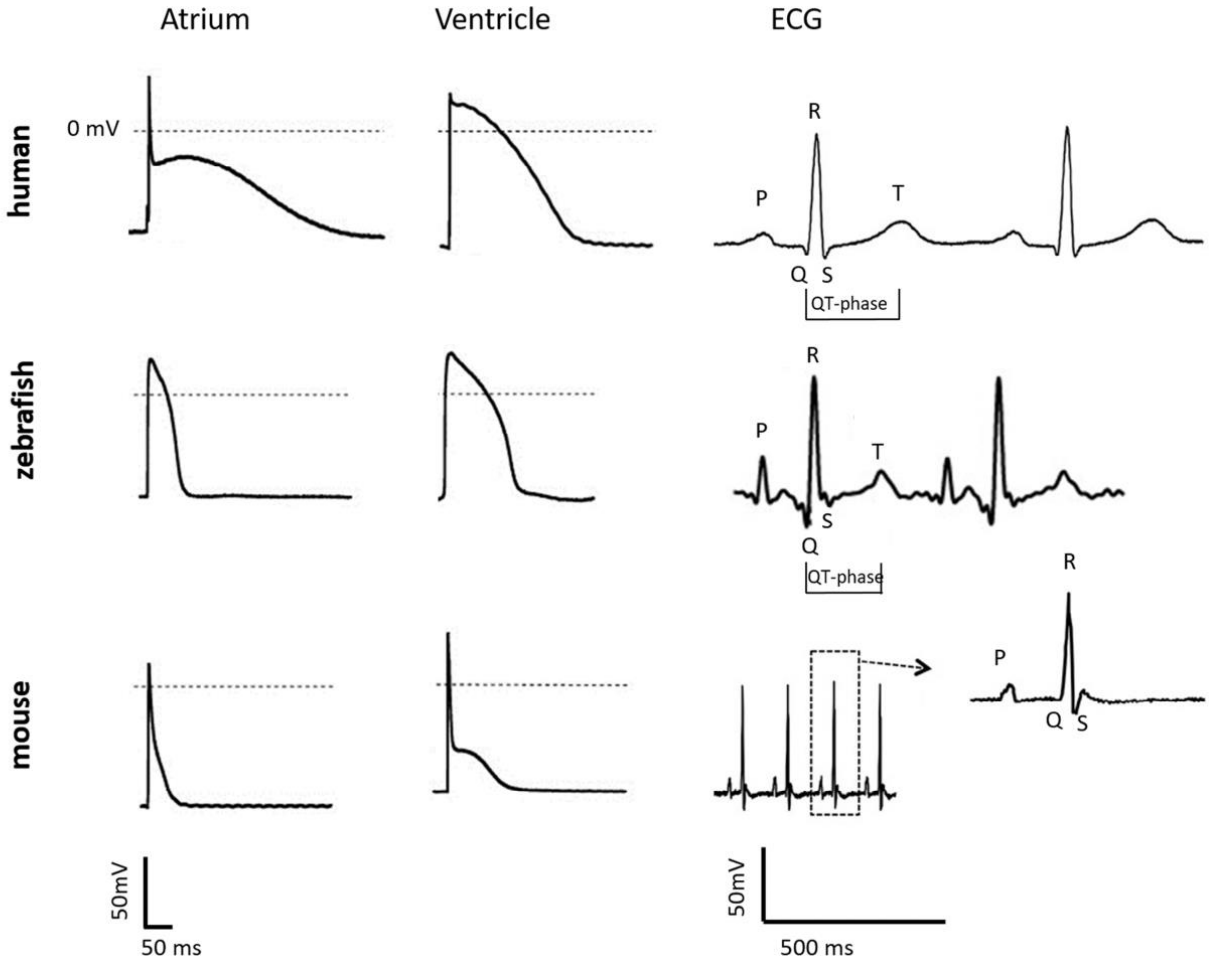


Fig. 2. Representative shapes of atrial (left column) and ventricular (middle column) cardiac action potentials (AP) and electrocardiograms (ECG, right column) from human, adult zebrafish and mouse.

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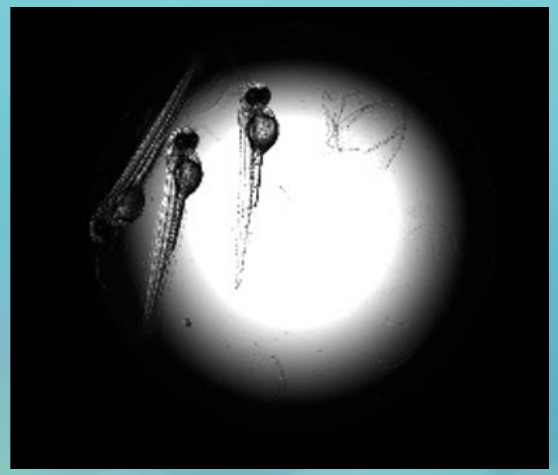
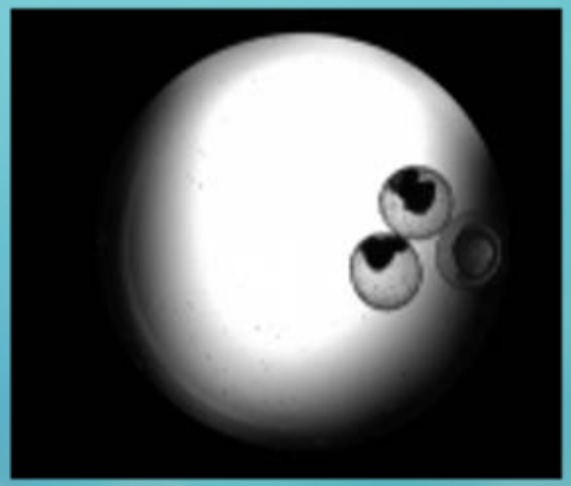
Zebrafish image analysis poses several challenges

- **Orientation**
  - Animals are not optimally positioned for recording
    - Garbage in, garbage out
  - Animal movement in response or reactions to injections or chemical compounds
- **Imaging parameters**
- **Data imbalance**
  - Obtaining animals with abnormal/disease-like characteristics can be challenging
    - Low yield & Toxic treatments
- **Objective:** develop an **orientation strategy** so that we can capture animals that have:
  - Consistent orientation
  - Do not move
  - Maximize number of animals captured





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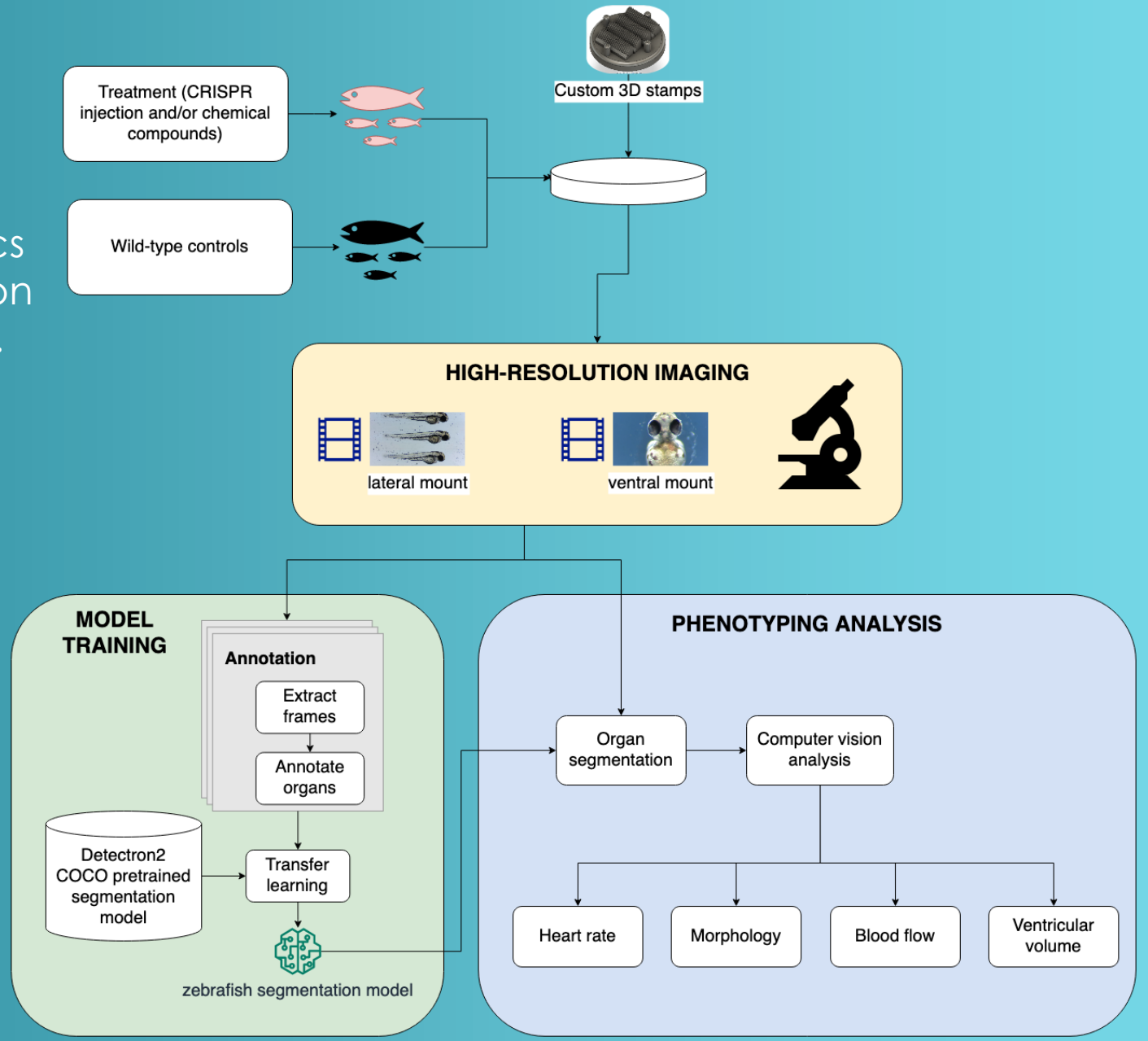
Methodology

Objective:

Quantify cardiac metrics to aid in the classification of cardiac-phenotypes.



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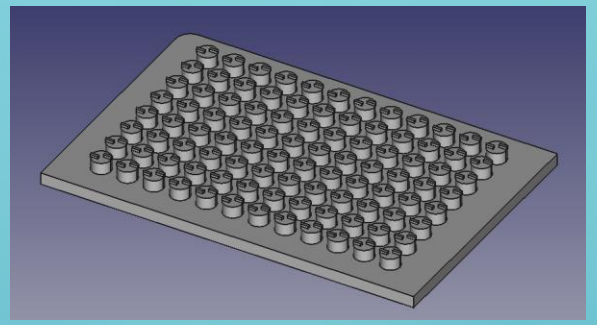
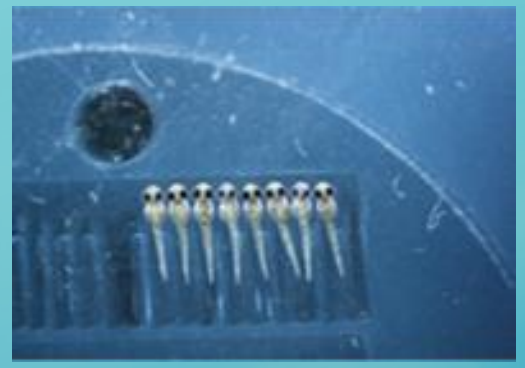
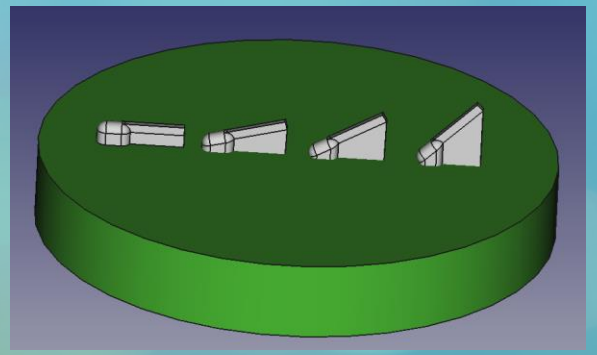
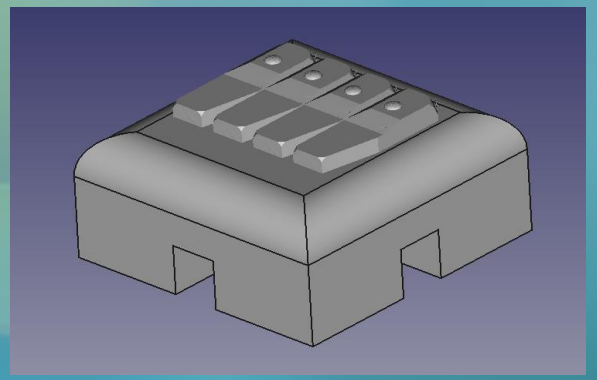
Designed 3D-printed stamps to standardize zebrafish orientation

- **Benefits**:
  - Consistent orientation
  - Do not move
  - Maximize number of animals captured
- Ventral
  - Zebrafish are mounted facing upwards
- Lateral
  - Zebrafish are mounted longitudinally









1.Wittbrodt JN, Liebel U, Gehrig J. Generation of orientation tools for automated zebrafish screening assays using desktop 3D printing. *BMC Biotechnol*. 2014;14:36.

2. Kleinhans DS, Lecaudey V. Standardized mounting method of (zebrafish) embryos using a 3

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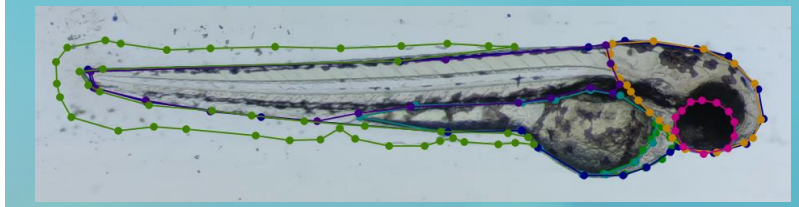
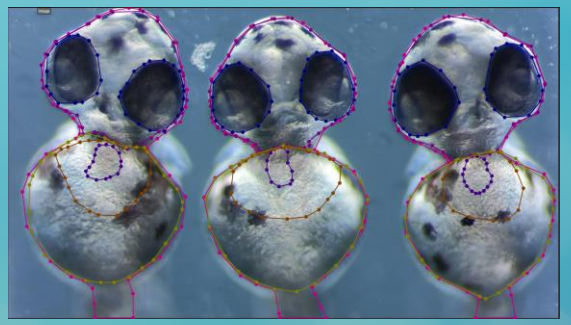
Using instance segmentation models to automate organ detection

- After image acquisition, we need a way to label the different organs of the animal.
  - **Doing this by hand would be very time-consuming!**
- Can we leverage pre-trained models to accelerate organ segmentation?
- Detectron2: objective detection and segmentation library by Facebook AI Research
  - Provides state-of-the-art detection and segmentation algorithms, pre-trained using COCO dataset.
- Selected pre-trained model: Mask-RCNN with ResNET-101+FPN backbone.

Generate labelled data (~2,400 images, nine organ labels)





Split train, validation, and test sets

Fine-tune instance segmentation model
(Mask-RCNN with ResNET-101+FPN backbone)

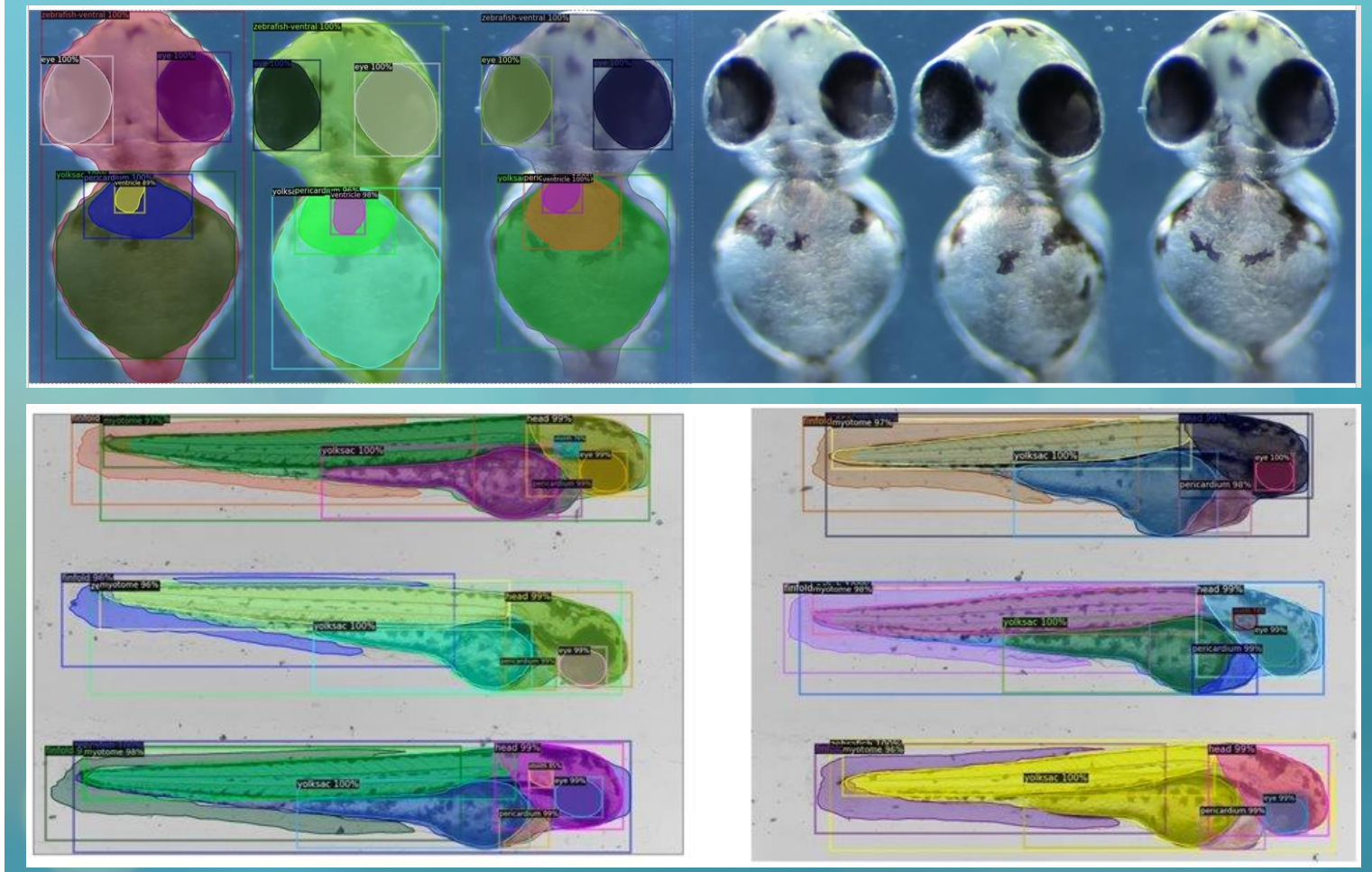
Instance segmentation model accurately detects major zebrafish organs

Objective:
Segment regions of the model organism which can later be used to quantify phenotypic parameters:

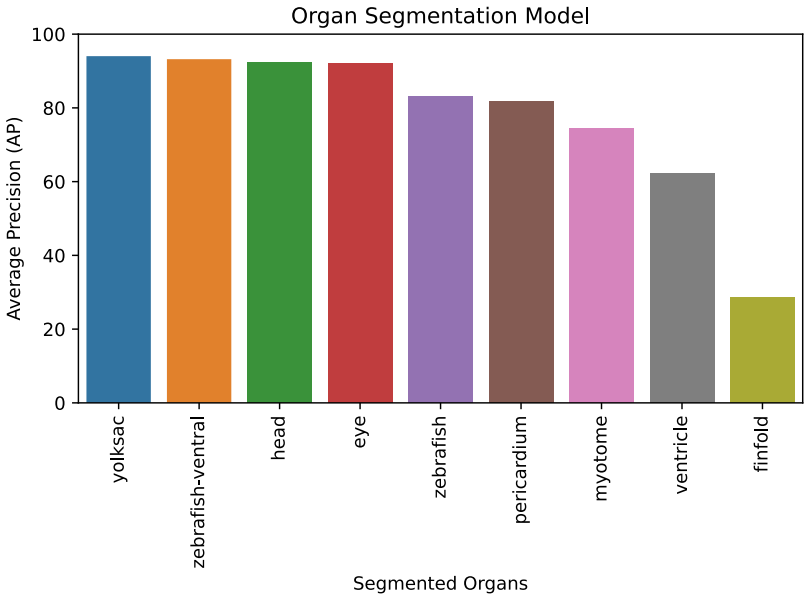
Cardiac Organ Segmentation Model's objective are to:

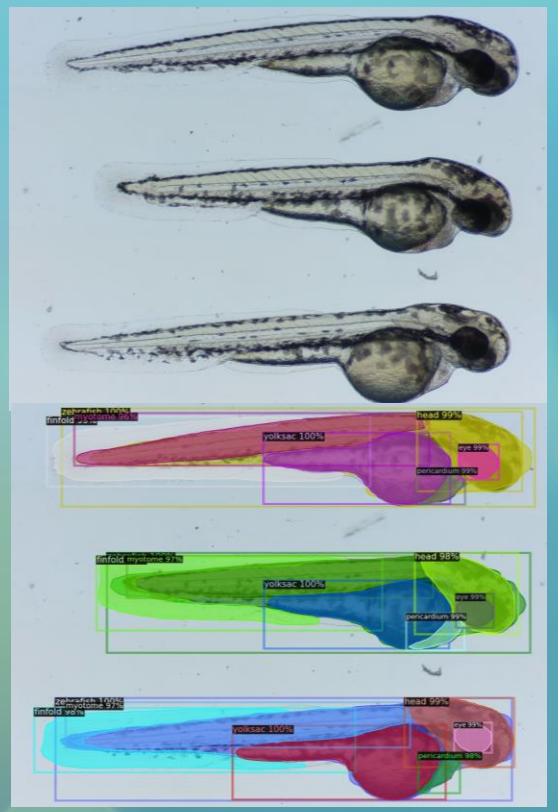
- Segment 9 classes depending on the view
  - **Ventral:** Ventricle, Zebrafish-Ventral
  - **Lateral:** Notochord, Myotome, Fin-fold, Zebrafish
  - **Common:** Eye, Head, Yolksac, Pericardium

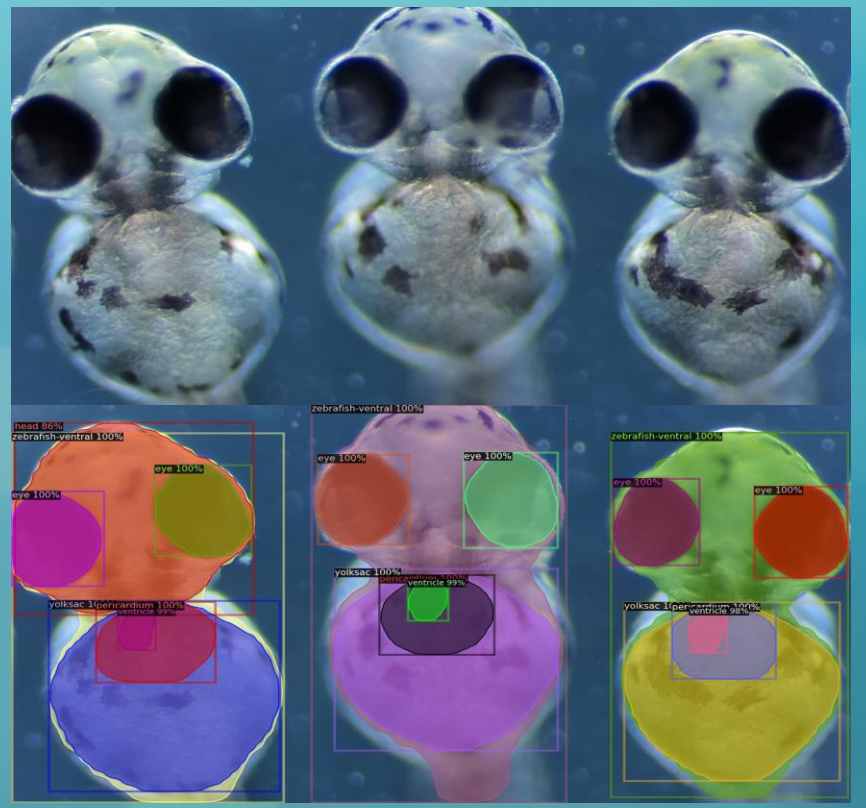
Cardiac **Parameters** include:
Heart Rate, Blood Flow, Ejection Fraction, Stroke Volume, Pericardium or Yolk Sac Edema



Organ segmentation model for automatic zebrafish detection







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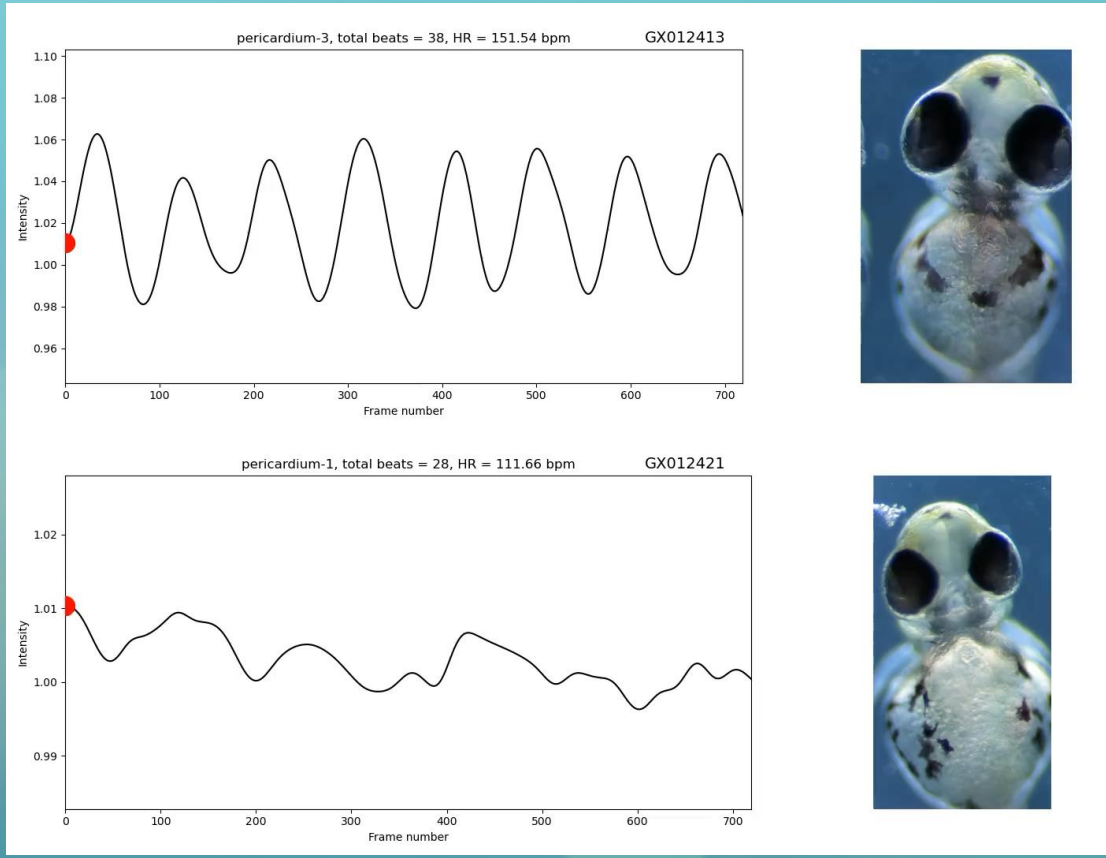
Our Overall Goal:

Use available image segmentation models +
Computer Vision/ Image Analysis in post-processing
to accelerate our cardiac-phenotypic analysis

Post-Processing

Using the organ segmentation results, images are post-processed using computer vision to accurately report out cardiovascular metrics.

After isolation of the pericardium, Fourier analysis is used to quantify heartrate.



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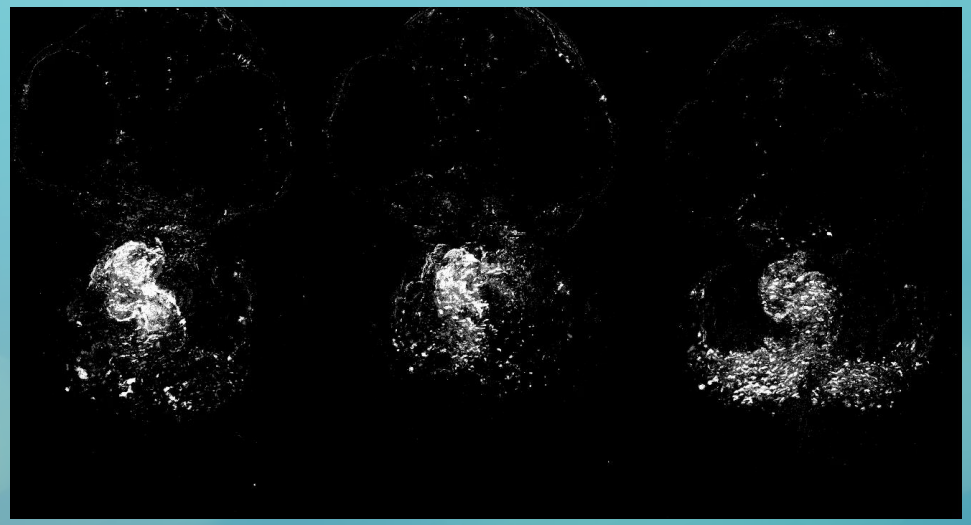
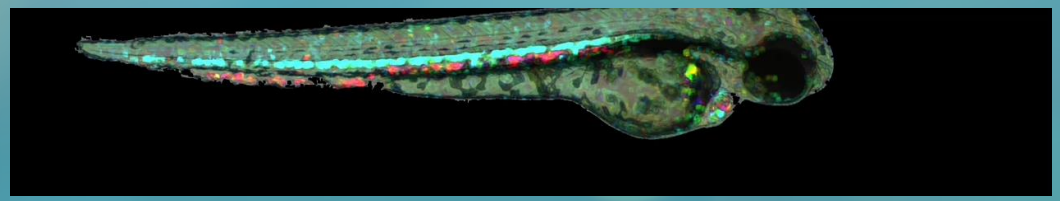
Blood flow

Visualize and quantify blood flow using background subtraction and optical flow techniques

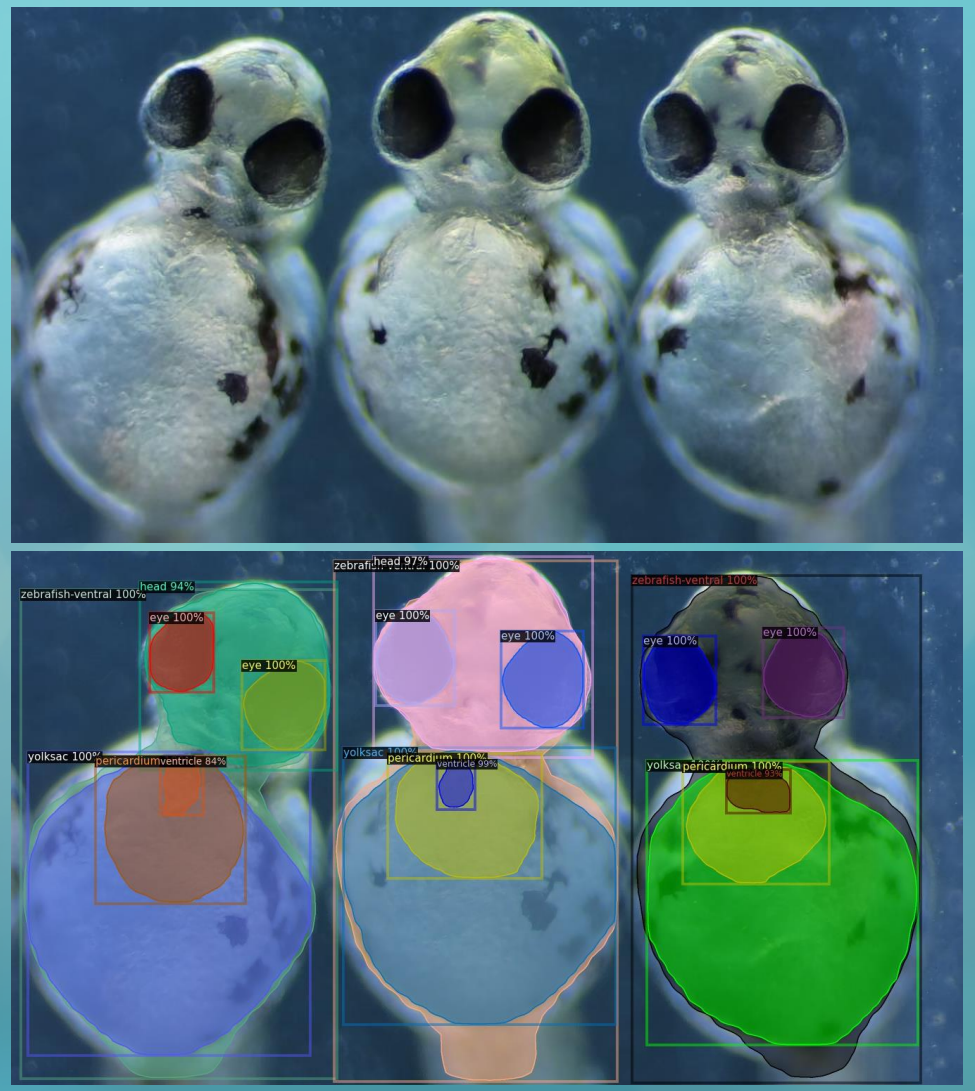




Ventricular volume

Using the organ segmentation results, to quantify edema-related phenotypes within pericardium and yolk sac regions

Ventricular Volume is the amount of blood flowing into the ventricle heart chamber.



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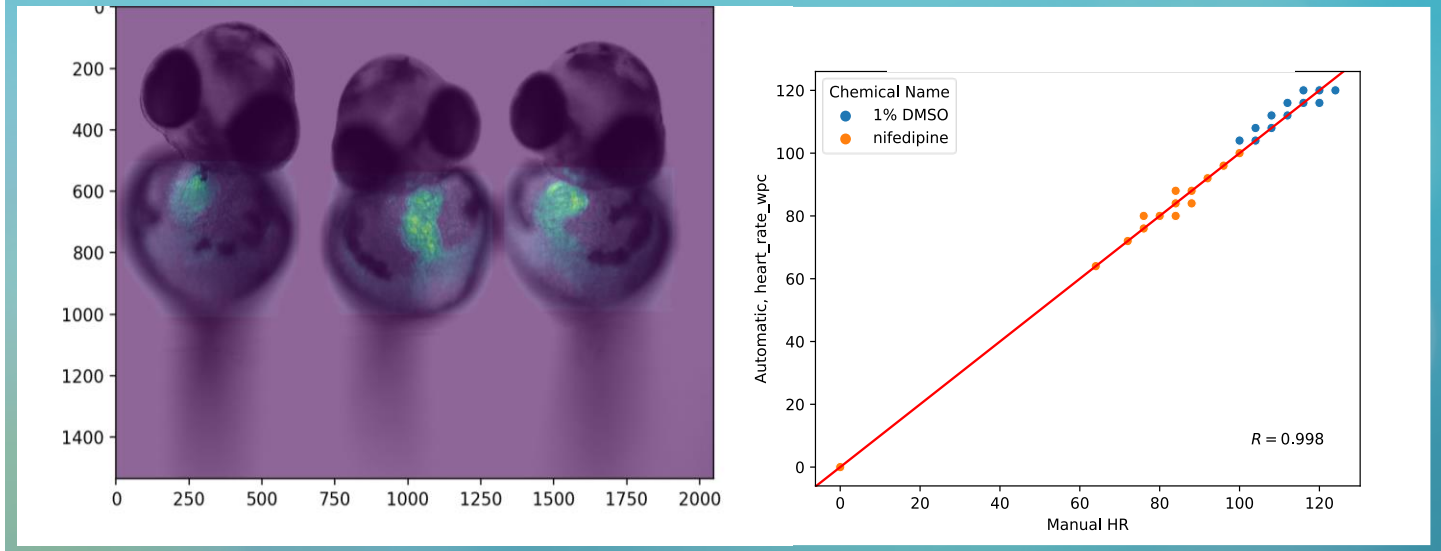
Results

Results

Multi-dimensional computer vision phenotypic analysis

Heartrates for each embryo were accurately quantified, for instance, when automatic and manual HRs were compared a correlation of 0.998 was achieved.



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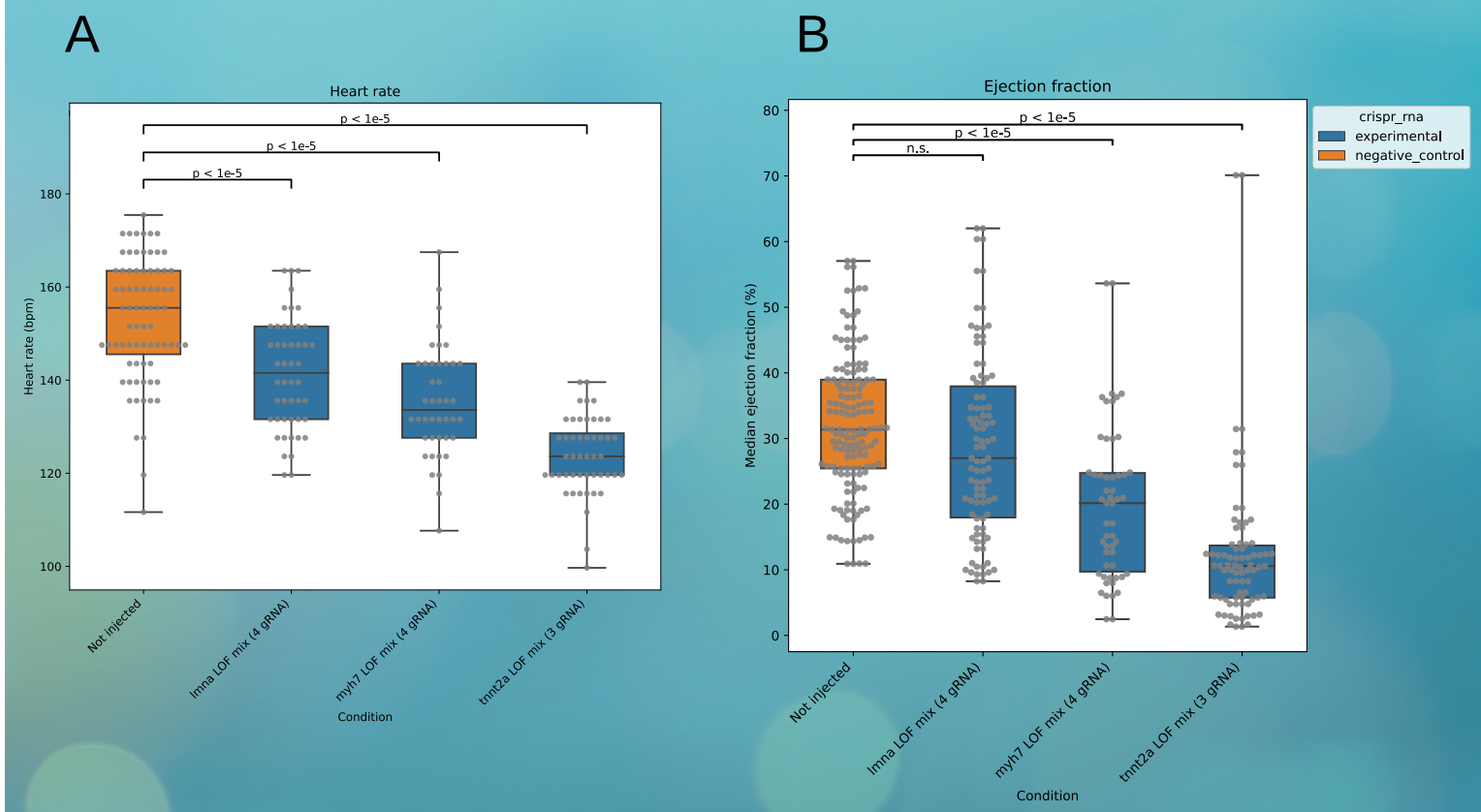
CRISPR loss-of-function screening of cardiovascular disease genes *myh7*, *lmna*, and *tnnt2a*

Results of cardiac metrics are analyzed by experts in the field, to ensure accuracy and validity.

- (A) Heart rates are significantly reduced in treatment groups; as well as (B) reduced ejection fraction for *myh7* and *tnnt2a.*



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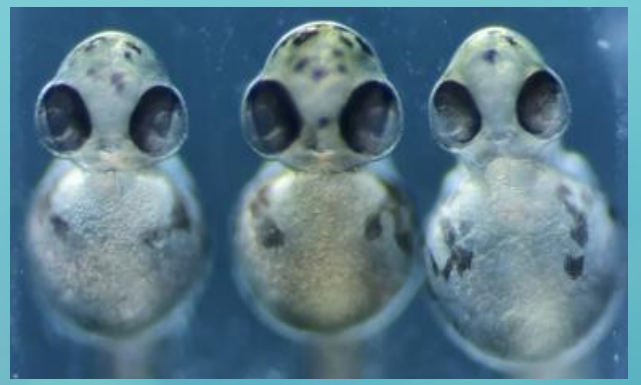
Results Applied

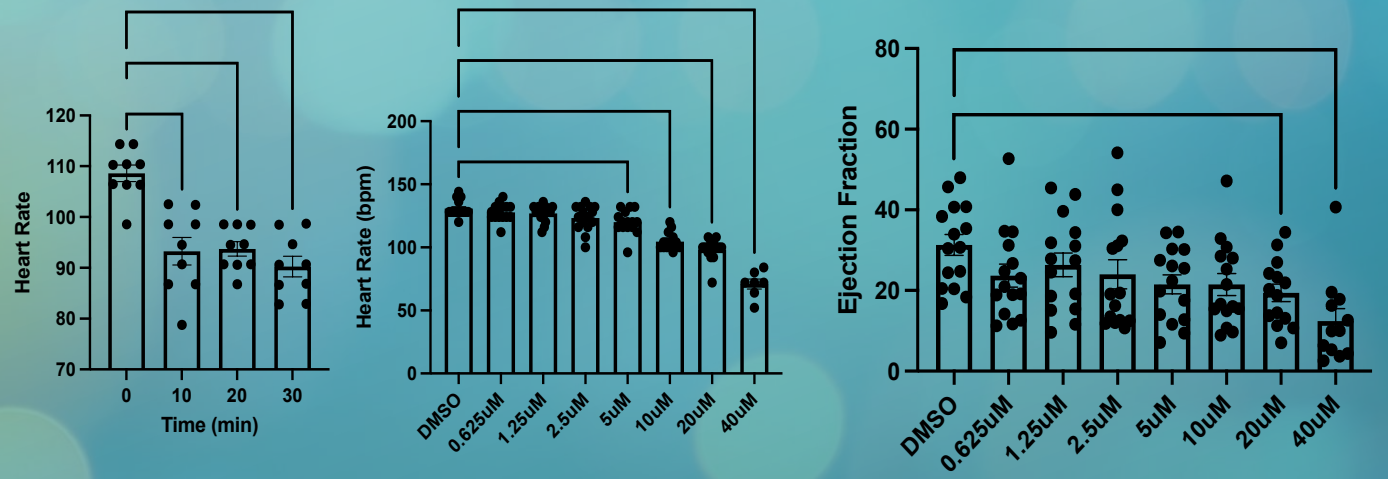
Multi-dose response experiments with cardiac compounds

Results of cardiac metrics are analyzed by experts in the field, to ensure accuracy and validity.

Treated zebrafish with **nifidepine**, a calcium channel blocker clinically used for treatment of angina and high blood pressure.

Observed expected cardiac phenotype: reduced heart rate, reduced cardiac output, and reduced ejection fraction.





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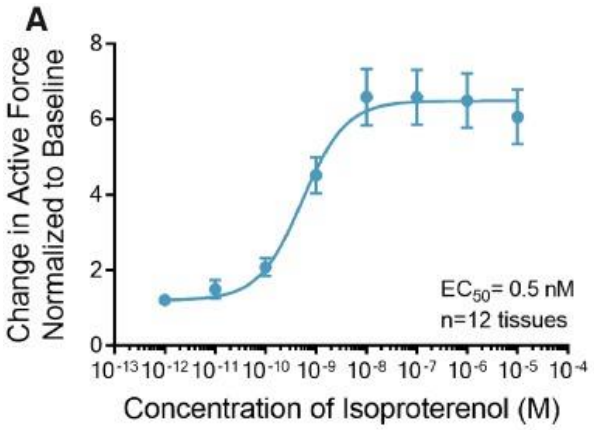
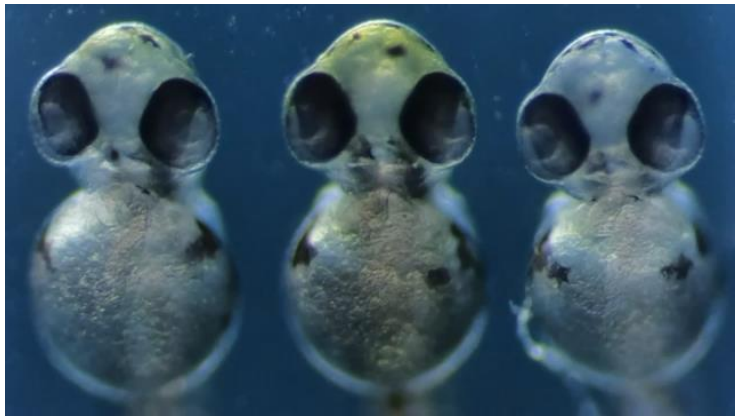
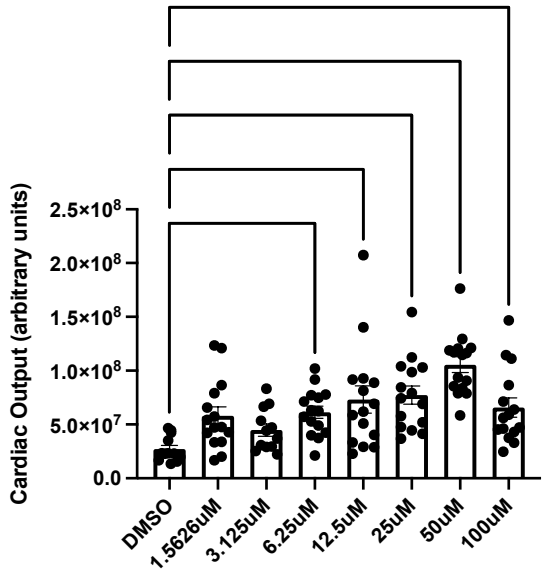
Our zebrafish model recapitulates human phenotypic cardiac responses to Isoproterenol

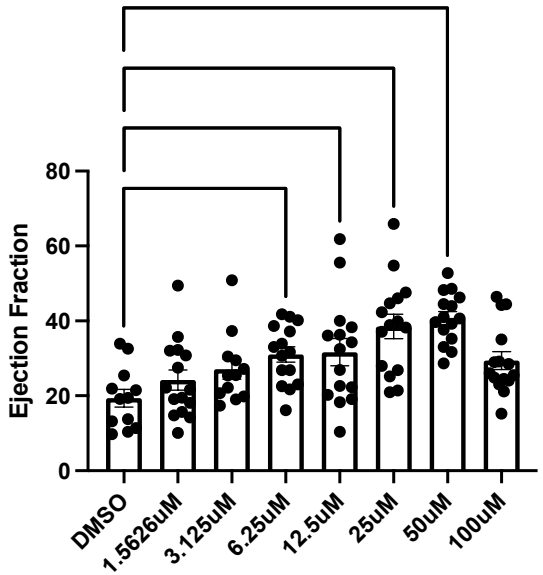
Clinical use	Treatment of bradycardia / heart block
Mechanism	Non-selective beta-adrenergic agonist
Half life	2.5-5 minutes
MW	211.258 g/mol
Expectation	Increased HR, CO, and EF
Observation	Increased HR, CO, and EF

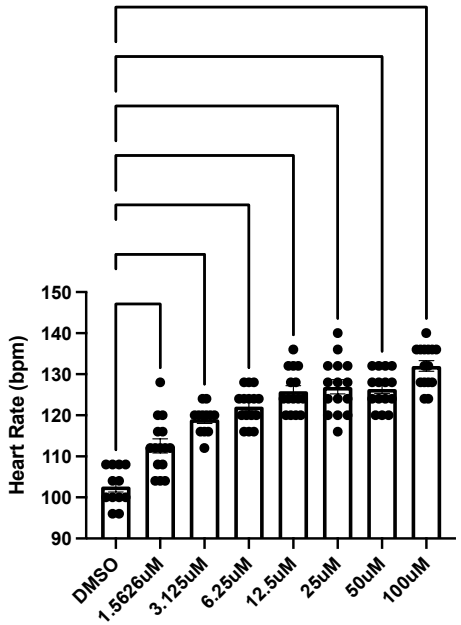


Published cell culture data









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Summary

Summary

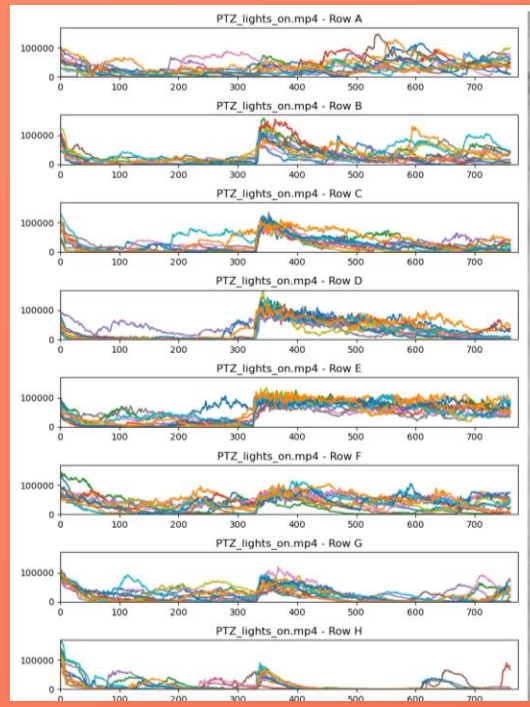
- Data variability is a large problem in live organisms, varying laboratory's. Imaging protocols must be standardized.
- Using machine learning-guided segmentations, the computer vision analysis process is automated and scalable to large volumes of experimental imaging data.
- Leveraging segmentation models accelerates the manual process of quantifying zebrafish phenotypes.
  - Turn around time significantly decreases from weeks or months to days.

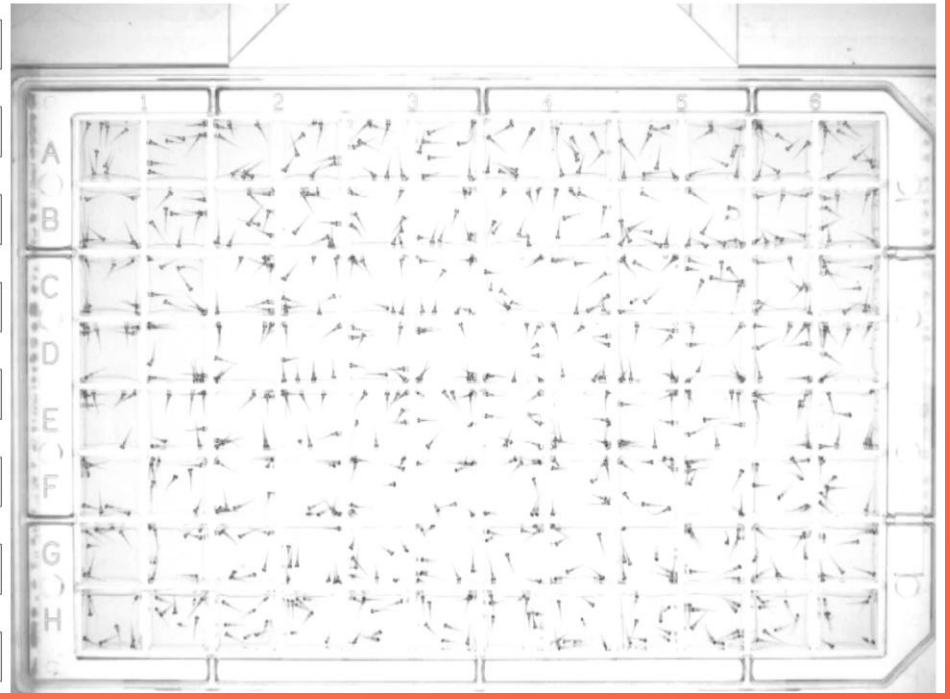
Behavioral/ Seizure Assay

Analyze Zebrafish in Epilepsy models.

Computer Vision Output:

- Behavioral features (seizure-like movement, velocity, activity)





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Thank you !

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Jonathan Volpatti
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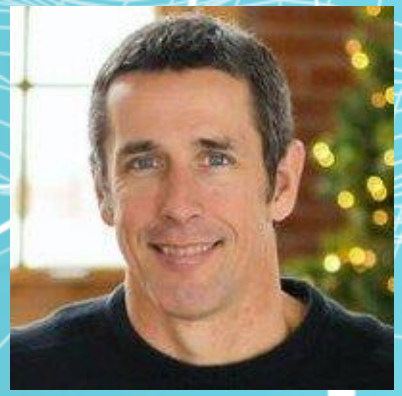

Steven Bishop
Senior Solutions Architect

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Q&A

Supplementary Slides

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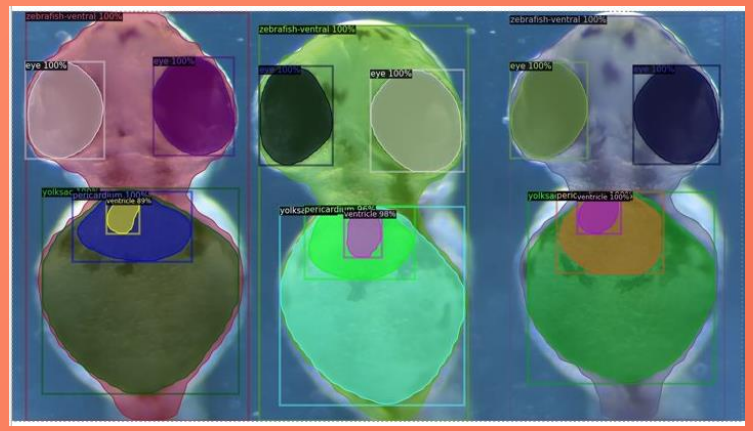
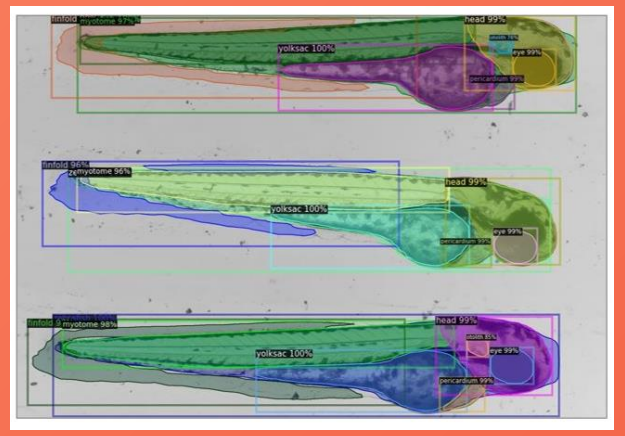
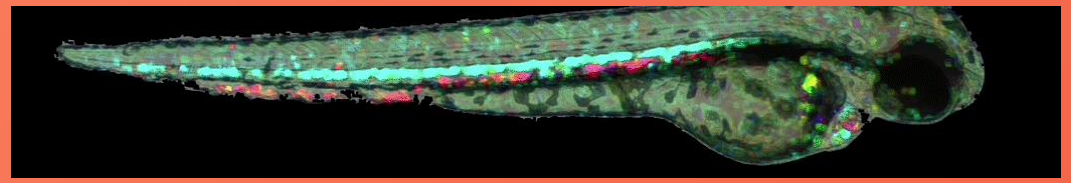
Speed to Validation with Computer Vision

Zebrafish offer whole organism vertebrate biology, where genetic predictions are rapidly validated *in vivo* on our automated high-throughput platform. Combined with our proprietary **Computer Vision software**, speed to target validation and Hit ID can be achieved in weeks.







Computer Vision Output:

- Morphological features (vascular or edema changes)
- Cardiac feature extraction (heart rate, ejection fraction)

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THERAPEUTIC AREA FOCUS & TARGET DISCOVERY PROGRAMS

Cardiometabolic

- Familial Dilated Cardiomyopathy
- Heart Failure (HFpEF)
- Hypertrophic Cardiomyopathy
- Myofibrillar Myopathy
- Atherosclerosis
- Non-Alcoholic Steatohepatitis

Neuroscience

- Epilepsy
- Parkinson's Disease
- Amyotrophic Lateral Sclerosis (ALS)
- Migraine
- Post-COVID-19 Anosmia

Rare Diseases

- Charcot-Marie-Tooth Disease
- Marfan Disease
- Alport Disease
- Polycystic Kidney Disease (ADPKD)

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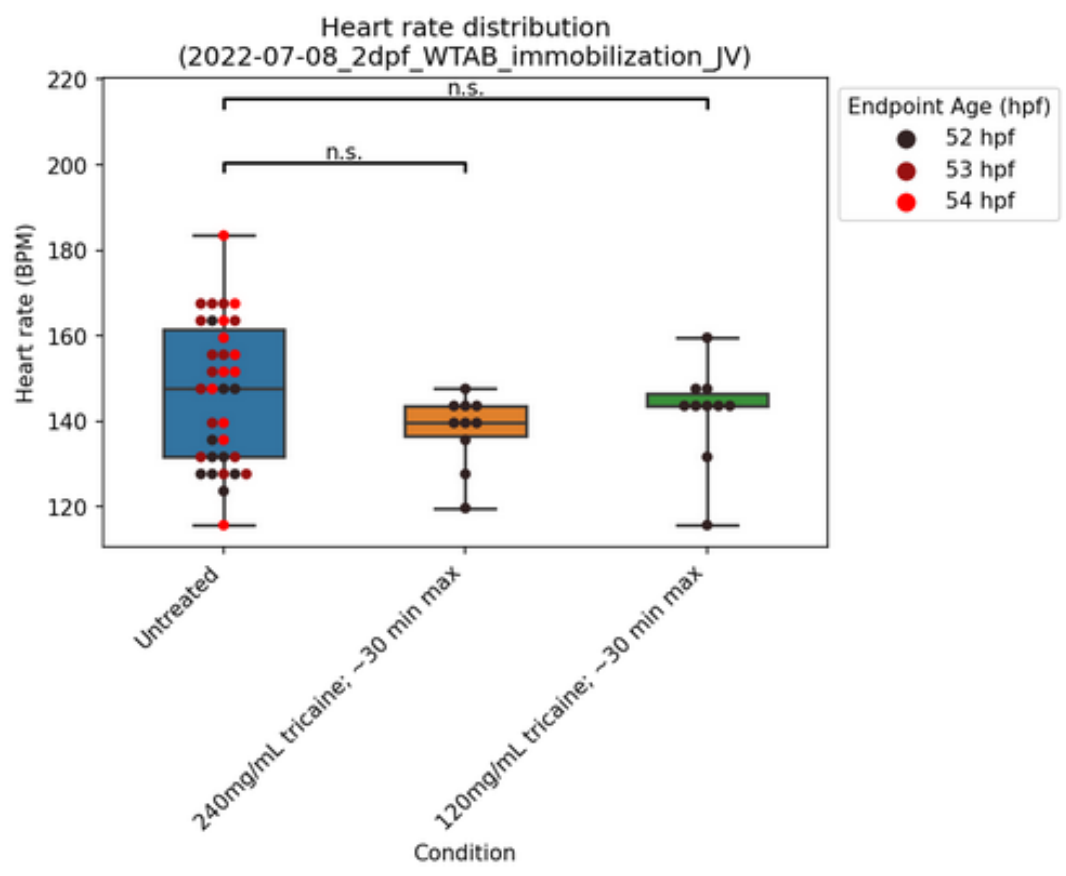
Ventricular Analysis

Effects of Tricaine

There were no significant differences in treatment versus untreated conditions (Student's t-test).



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