Automated High-Throughput Classification of Zebrafish Developmental Stages Using a Wide Field-of-View Gigapixel Microscope and Neural Network

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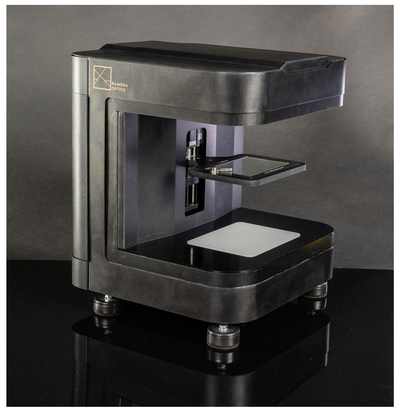
BACKGROUND AND MOTIVATION

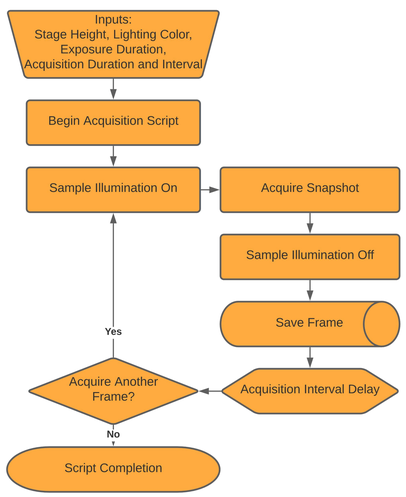
- *Danio rerio* (Zebrafish) are commonly used as a model organism.
- Automated recognition is a useful tool for phenotypic screening of morphology, gender and developmental stage.
- The Falcon Micro Camera Array Microscope (MCAM™) enables high throughput video capture and machine-learning assisted analysis.
- Wide field-of-view acquisition monitors 96 zebrafish simultaneously.
- Monitoring developmental stages with temporal precision will allow for automated screening and accurate, reproducible stimulus introduction at specific timepoints.

ACQUISITION DEVICE: FALCON MCAM™



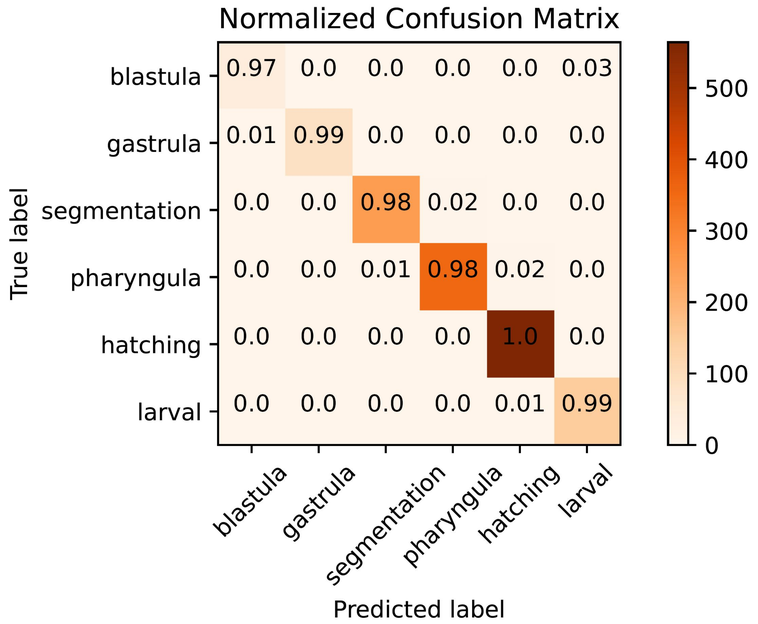
- Falcon Micro Camera Array Microscope (MCAM™)
- 0.7-gigapixel image acquisition
- Video capture frame rate configurable to 200 fps
- 8 x 12 centimeter field-of-view
- < 10-micron resolution
- Digitally controlled transmission and reflection lighting
- RGB and IR capabilities
- High-throughput simultaneous screening of multiple organisms
- Integrated machine learning analysis software in development

TIME-LAPSE ACQUISITION



- Autonomous acquisition with user defined parameters. This study used: 75 ms exposure, 1 minute acquisition interval, transmission illumination, RGB color image capture.
- Each 700 MB image is saved directly to the hard drive.
- A delay sets the image acquisition rate.
- During this delay the system has time to execute real-time data analytics or other custom functions.
- Stimulus introduction or other actions can be temporally coupled with zebrafish development.
- Total dataset file size: 4 TB

CNN PREDICTION RESULTS



99.2% prediction accuracy among all classes.

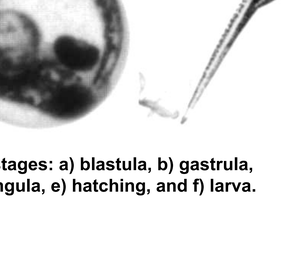
- Prediction inference takes just 4 seconds for the entire 96-well plate.
- All prediction deviations (except one) occur between adjacent stages.
- High confidence requirements yield accurate predictions.
- This is a successful proof-of-concept for future real-time classification tasks.

OBJECTIVE

The goal of this work is to design, build, and test a machine learning, neural network prediction workflow for autonomous real-time classification of zebrafish by developmental stage in a 96-well plate.

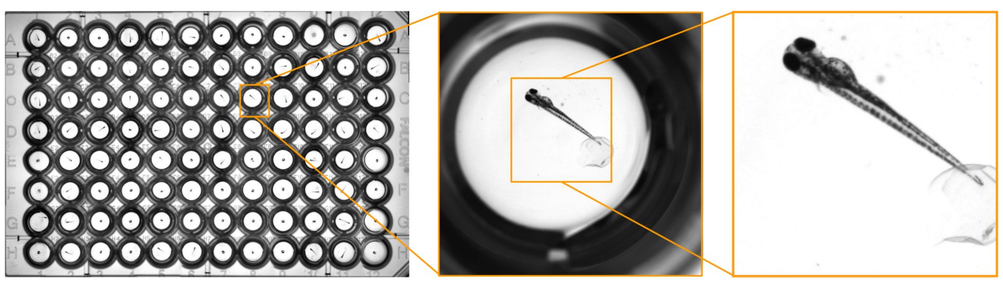
NETWORK ARCHITECTURE

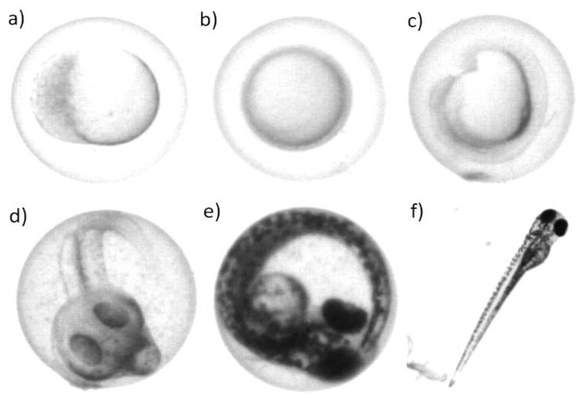
- Input accepts 600 x 600 pixel images.
- Convolution layers use filters to detect features in the input image.
- The weights of these filters are "learned" during model training.
- Max Pooling layers downsample the feature maps summarized by the previous layers.
- Flattening merges all layers into a 1-D array.
- The dense connection layer exploits all possible relationships of input nodes regardless of their spatial ordering.
- The Softmax output layer classifies each input by selecting the highest confidence prediction.
- This architecture has 8,659,398 trainable parameters.
- Different numbers of convolution layers were compared, and this architecture yielded the highest accuracy.



EXPERIMENTAL PROCEDURE

1) 96-well plate of recently fertilized zebrafish embryos ~3 hours post fertilization
2) 100-hour time-lapse acquisition resulting in 5,804 frames
3) Each frame was split into 96 rectangular images and cropped to 600x600 pixels
4) 5 wells were targeted for dataset labeling = 29,020 images
5) Each image was labeled one of 6 stages of embryonic development
6) The dataset was divided for model input: 75% training, 20% validation, 5% test
7) Multiple convolutional neural networks (CNN) were trained and tested





Zebrafish developmental stages: a) blastula, b) gastrula, c) segmentation, d) pharyngula, e) hatching, and f) larva.

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