Machine Learning Controlled Zebrafish Assay Pipeline Enabled by Developmental Classification and a Wide Field-of-View Gigapixel Microscope

John Efromson[1,2], Thomas J.J. Doman[1], Maxwell Zheng[1], Ron Appel[1], Mark Harfouche[1],
Gregor Horstmeyer[1], Roarke Horstmeyer[1,3], Chau-Wai Wong[2]
[1]Ramona Optics, Durham, NC [2]North Carolina State University, Raleigh, NC [3]Duke University, Durham, NC

BACKGROUND AND MOTIVATION

- *Danio rerio* (Zebrafish) are commonly used as a model organism.
- Automated recognition is a useful tool for phenotypic screening of morphology, developmental stage and disease states.
- 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 allows for automated screening and assay manipulation at specific time points.

OBJECTIVES

- Design, build and test a machine learning, neural network prediction workflow for autonomous real-time developmental classification.
- Trigger a behavioral motion tracking assay at a specific time point in the zebrafish life cycle using this autonomous screening.
- Monitor experimental progress remotely by email notification including high quality images and classification summary.
- Capture quantified motion tracking data across multiple days of the larval stage for further behavioral analysis.

REMOTE MONITORING BY EMAIL

gigapixel.MCAM.bot.2021@gmail.com Sat, Aug 14, 3:18 AM

to me

Greetings!

I'm still watching your fish for you!

59 have hatched.

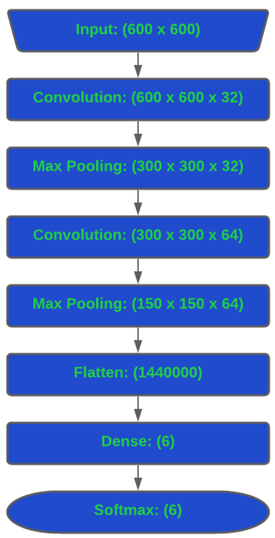
Hatched fish include: ['A1', 'A3', 'A4', 'A7', 'A8', 'A12', 'B1', 'B2', 'B4', 'B8', 'B9', 'B10', 'C3', 'C4', 'C7', 'C8', 'C9', 'C10', 'C11', 'D1', 'D2', 'D5', 'D7', 'D9', 'D10', 'D12', 'E1', 'E4', 'E5', 'E7', 'E8', 'E9', 'E11', 'F1', 'F2', 'F4', 'F6', 'F7', 'F8', 'F9', 'G2', 'G3', 'G4', 'G5', 'G6', 'G7', 'G9', 'H1', 'H2', 'H3', 'H4', 'H5', 'H6', 'H7', 'H8', 'H9', 'H10', 'H11', 'H12'].

ACQUISITION DEVICE: FALCON MCAM™

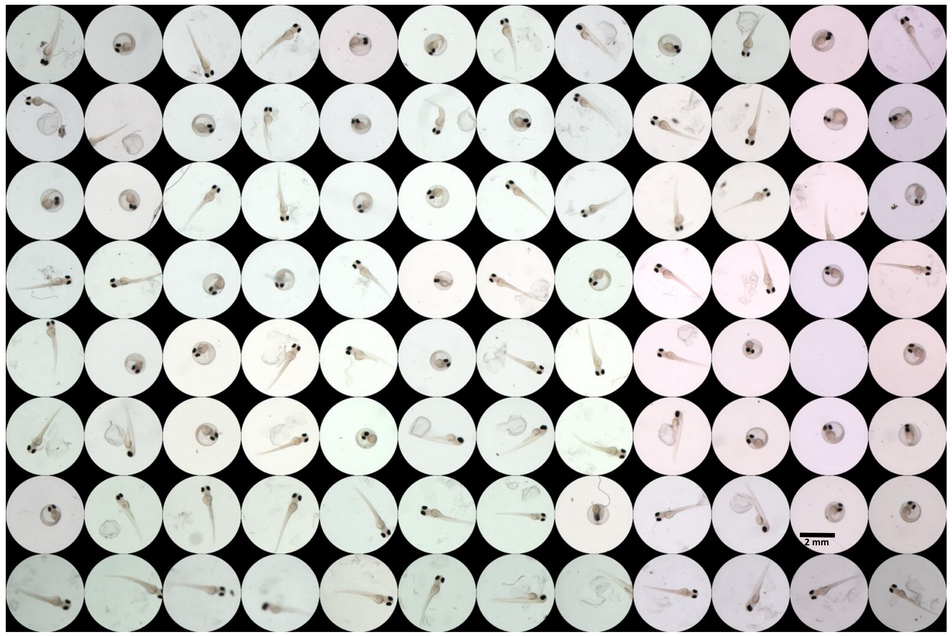
- Falcon Micro Camera Array Microscope (MCAM™)
- 54 cameras combined form 0.7-gigapixel images
- Video capture frame rate configurable to 260 fps
- 8 x 12 centimeter field-of-view
- 6-micron per pixel resolution
- Digitally controlled transmission and reflection lighting with RGB and IR capabilities
- High-throughput simultaneous screening of multiple organisms
- Integrated machine learning analytics software

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.
- We previously demonstrated the use of this architecture to obtain >99% prediction accuracy of development.
- This model was trained with 51,489 images classified as embryo or larva.



DEVELOPMENTAL CLASSIFICATION AND TRIGGERED TRACKING ASSAY



TIME-LAPSE DEVELOPMENTAL CLASSIFICATION

- A time-lapse acquisition captures one frame every five minutes, and the autonomous classifier makes a prediction of all 96 fish in under two minutes.
- The transition threshold is set to sixty-four hatched larvae to trigger motion tracking.
- Every two hours an email notification is dispatched summarizing the experimental progress including high quality images of the well plate, as shown above, to allow for manual confirmation if desired.
- Remote monitoring continues over night and on weekends freeing up valuable time.
- 97% prediction accuracy during the hatching event that triggers the second stage of this automated assay workflow.
- Higher prediction accuracy was seen for larvae than embryos resulting in accuracy improvements over the course of the experiment and critically at the time point of interest when two thirds of the well plate have hatched.

RECURRENT VIDEO MOTION TRACKING

- Motion tracking triggers when two thirds of the 96-well plate have hatched as defined by the user's input threshold.
- Thirty second videos are acquired every fifteen minutes for the remainder of the five-day experiment at approximately fifteen frames per second.
- A machine learning tracking algorithm automatically detects the movement of all 96 zebrafish in approximately ten minutes.
- Movement patterns are graphically displayed as seen above.
- Velocity, displacement and other metrics are calculated and output for further analysis.

Contact: john@ramonaoptics.com

NSF NIH Duke NC STATE ramona