ramona

AI/ML in Biomedical Research with Zebrafish and a Multi-Camera Array Microscope (MCAM™)

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Ramona Optics, Durham, NC
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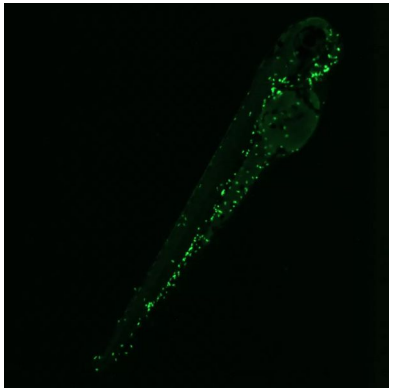
Toxicology with Zebrafish and Big Data Challenges

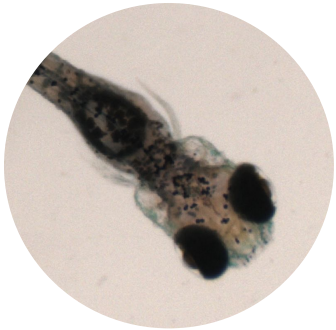
- Zebrafish
- Multi-Camera Array Microscope (MCAM™)
- Applications and Algorithms in Biomedical Research / Computer Vision
- Synthesis from Big Data

Zebrafish as a Model Organism

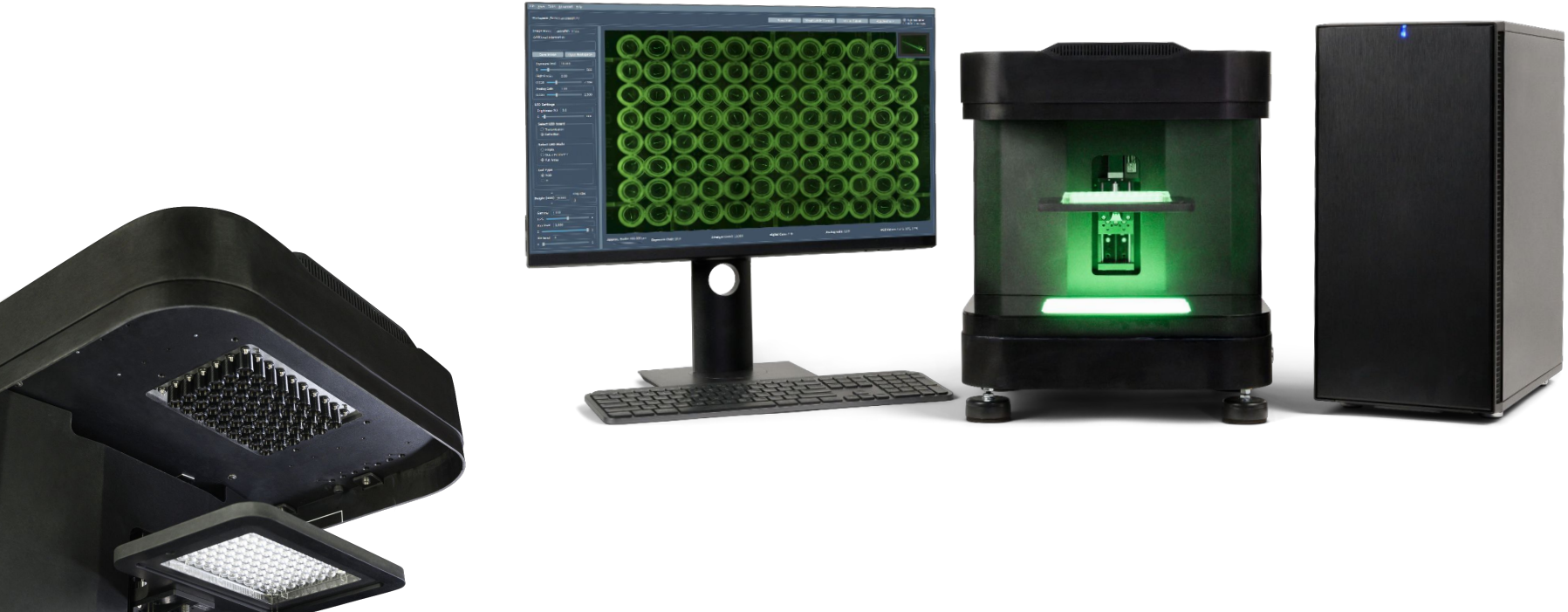
- Transparent vertebrate
- Cardiovascular, immune, and neural development in 7 days
- Distinct behavior and morphology
- 70% genetic homology with humans
- Common use in toxicology and pharma research



- Easy breeding
- Large clutch size
- Easy maintenance
- Economical



Multi-Camera Array Microscope (MCAM™)

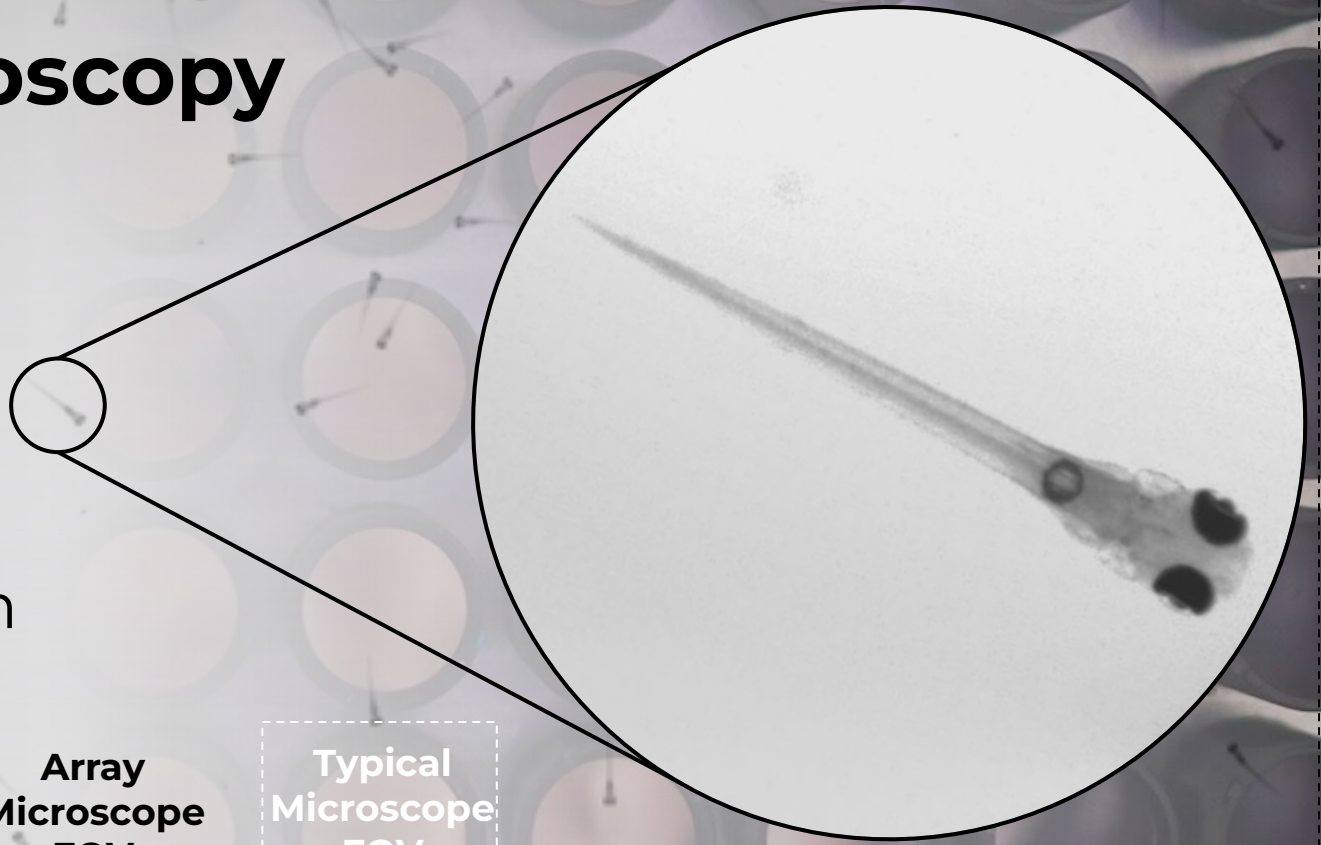


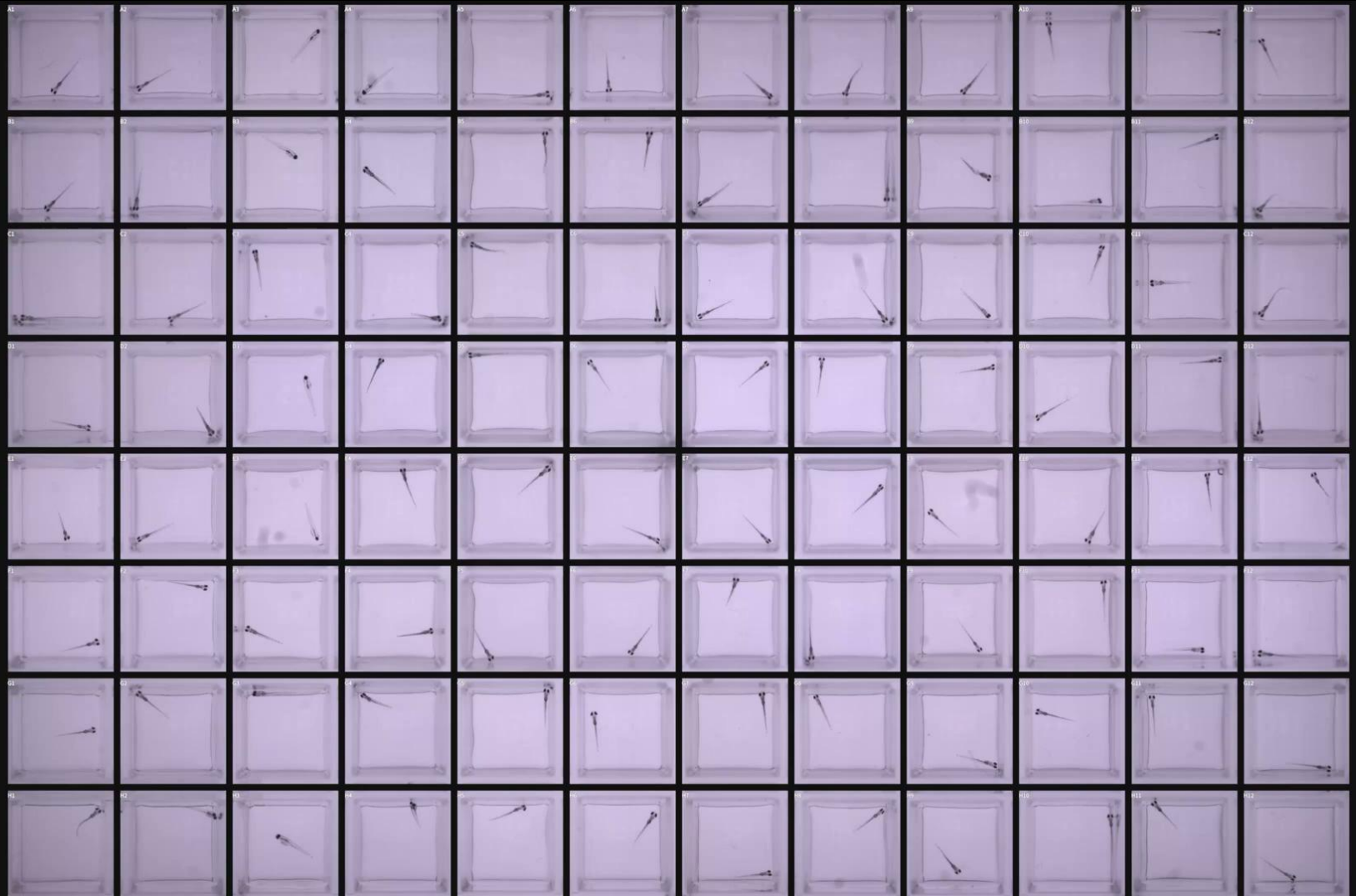
Array Microscopy

Large Area
SBS Plate
(120 x 85mm)

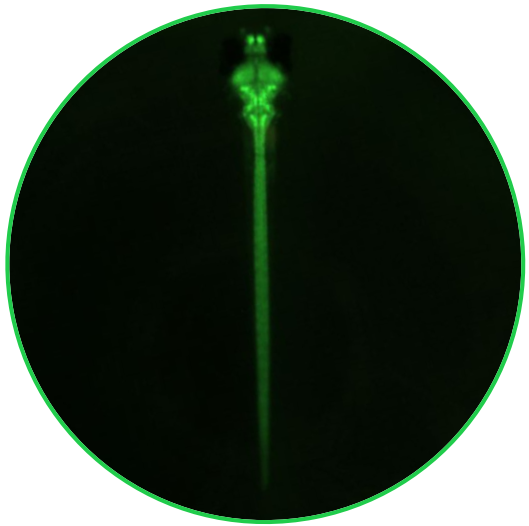
+

High Resolution
3µm - 10µm



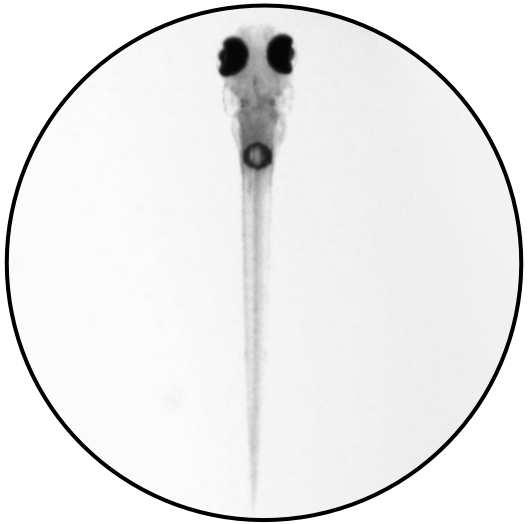


Imaging and Analysis Modalities



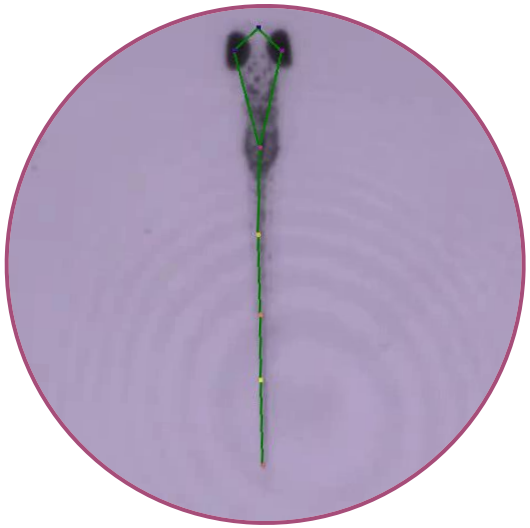
Physiology

GFP+RFP



Morphology

VISIBLE



Behavior

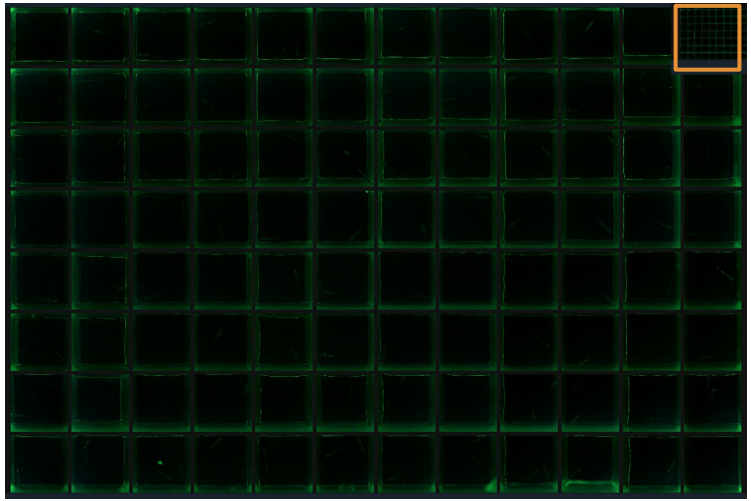
INFRARED

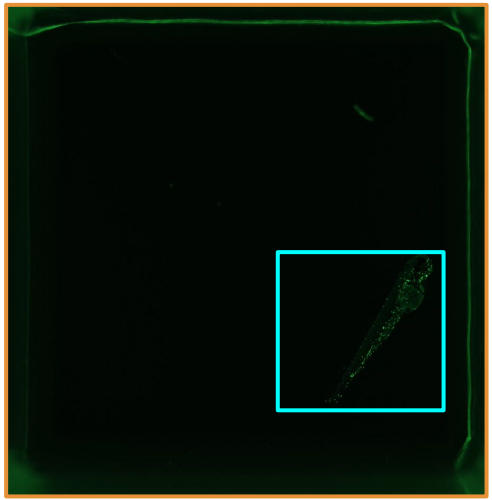
Workflow | **ImmunoToxicology**

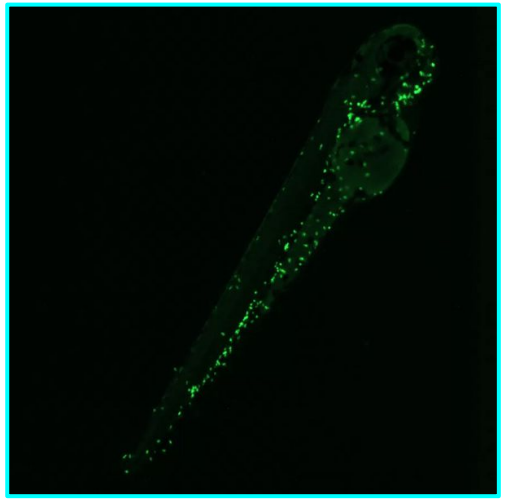
Disease Modeling

Assay: Frames per **hour**

Process: bulk and counting



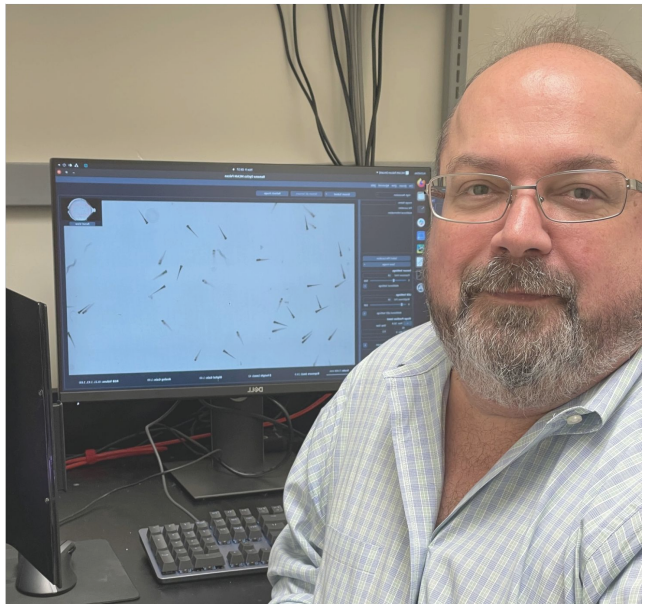




Line: *lyz:EGFP* [120 hpf]

Source: NC State University

ImmunoTox | **Yoder Lab, NCSU**



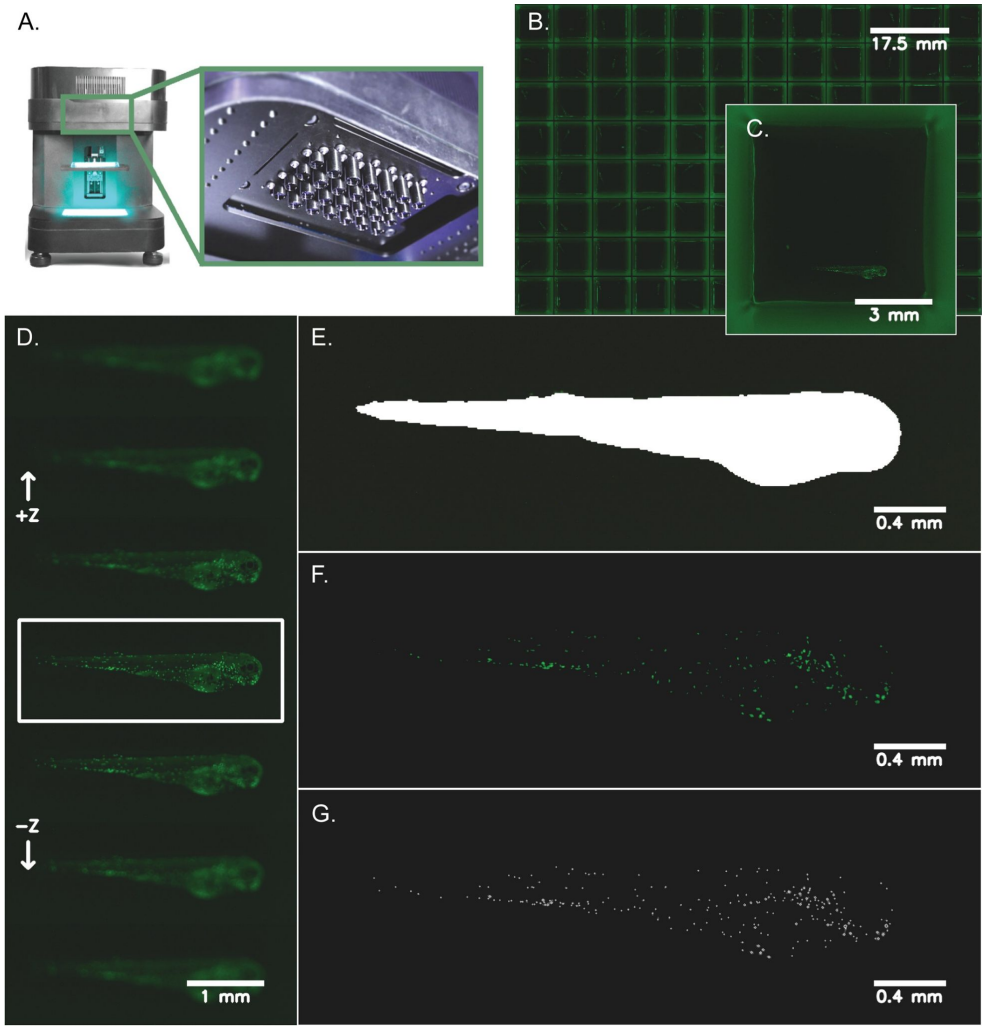
PLOS ONE

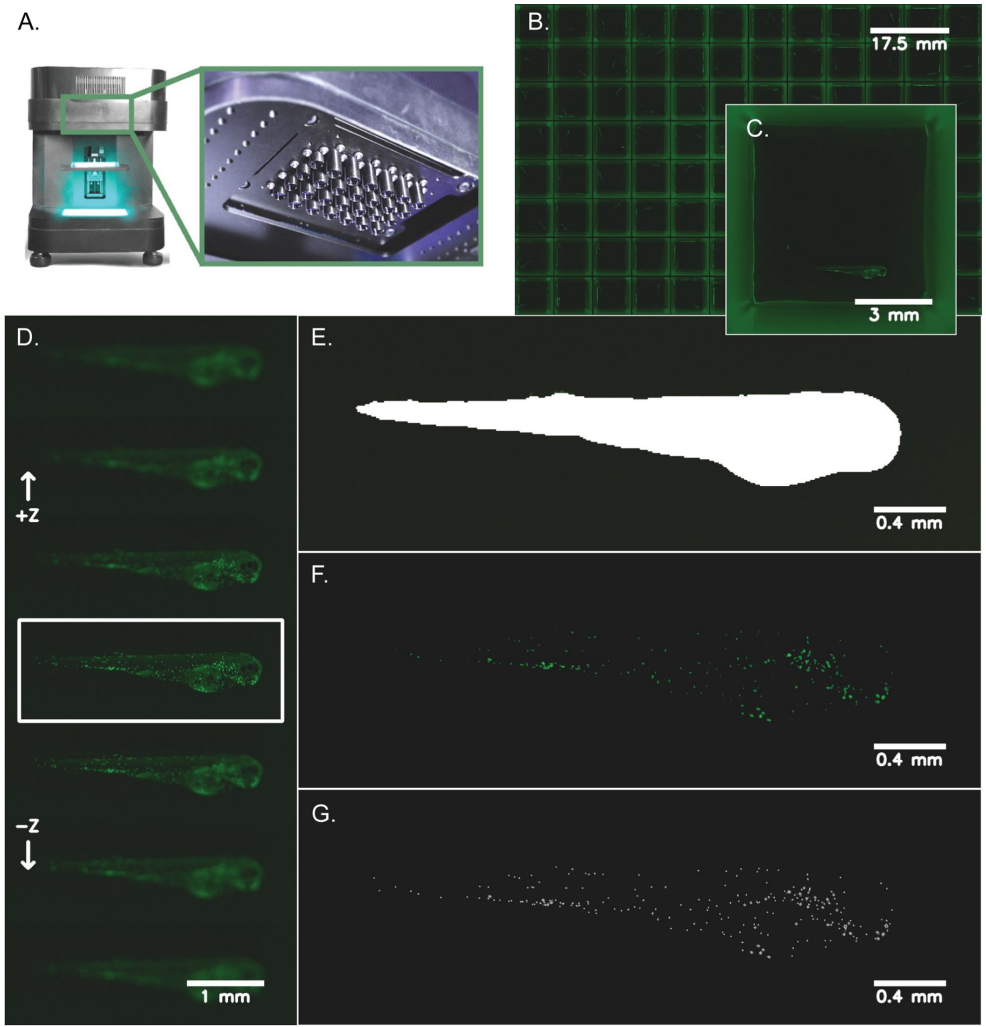
RESEARCH ARTICLE

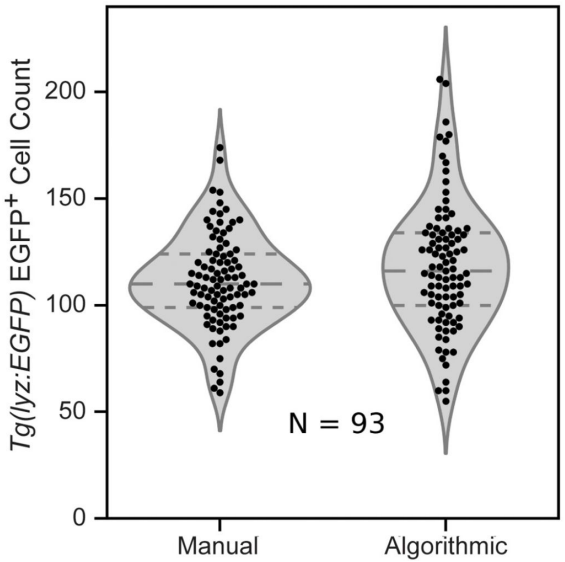
Automated, high-throughput quantification of EGFP-expressing neutrophils in zebrafish by machine learning and a highly-parallelized microscope

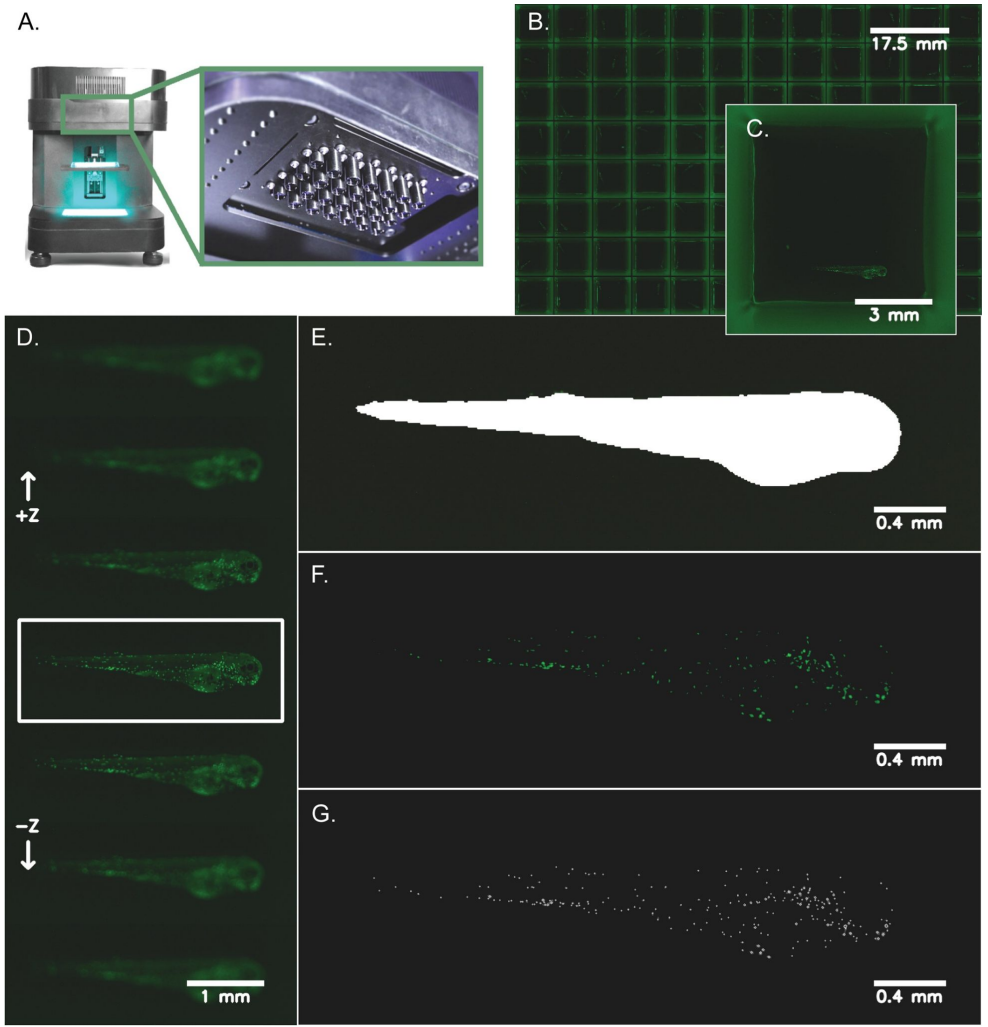
John Efromson[1]*, Giuliano Ferrero[2], Aurélien Bègue[1], Thomas Jedidiah Jenks Doman[1], Clay Dugo[1], Andi Barker[2], Veton Saliu[1], Paul Reamey[1], Kanghyun Kim[3], Mark Harfouche[1], Jeffrey A. Yoder[2]*

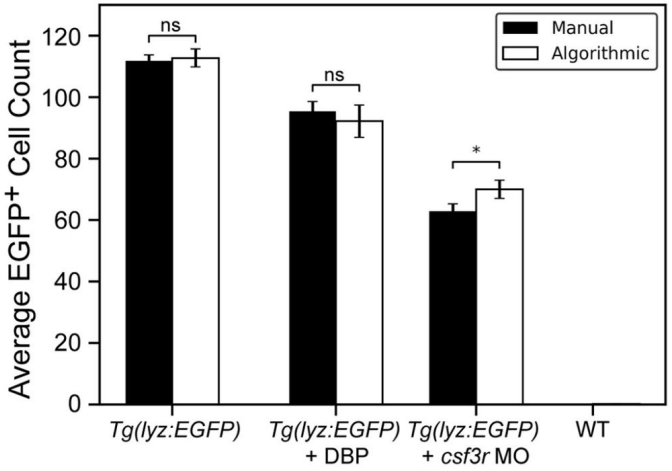
1 Ramona Optics Inc., Durham, NC, United States of America, **2** Department of Molecular Biological Sciences, North Carolina State University, Raleigh, NC, United States of America, **3** Department of Biomedical Engineering, Duke University, Durham, NC, United States of America

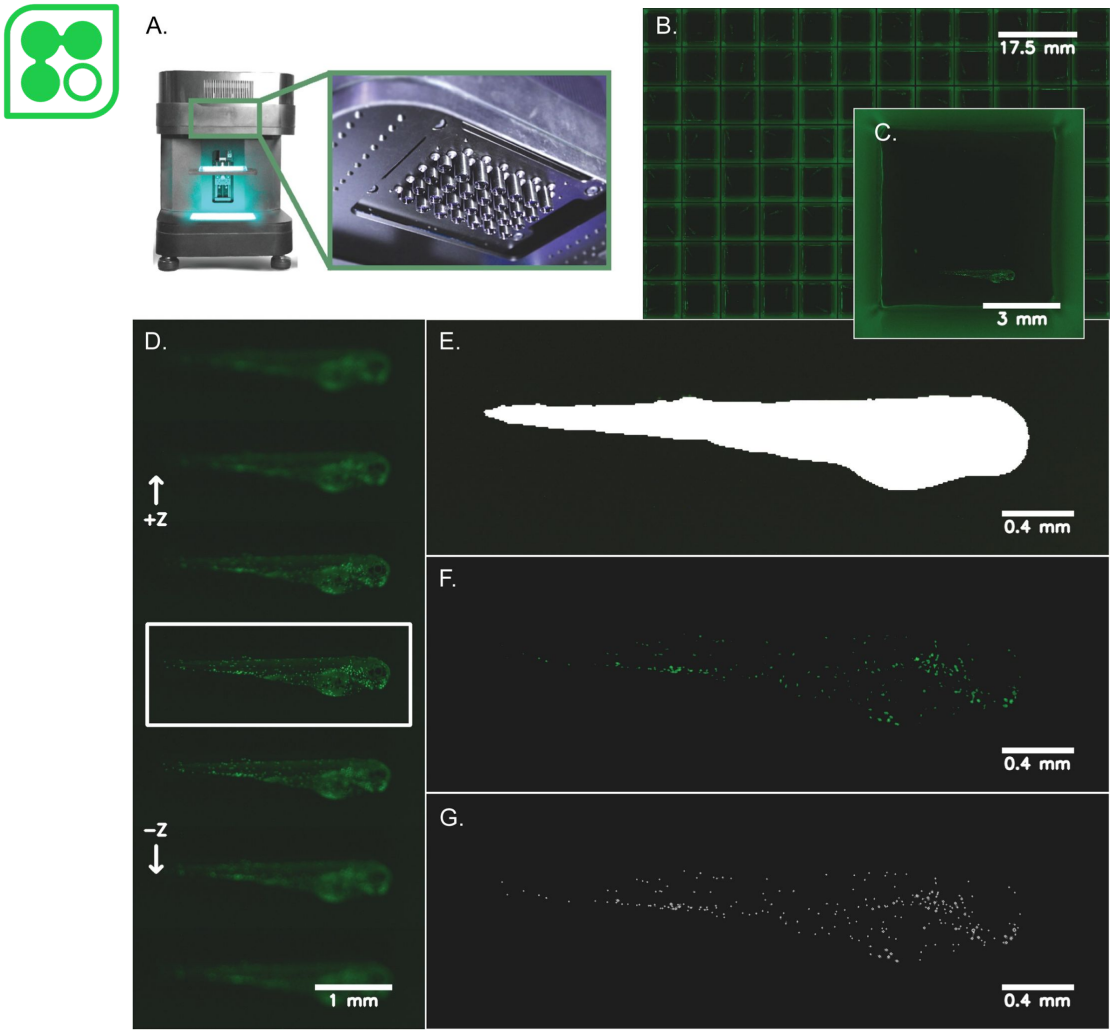

A.
B.
17.5 mm
C.
3 mm
D.
+z
−z
1 mm
E.
0.4 mm
F.
0.4 mm
G.
0.4 mm

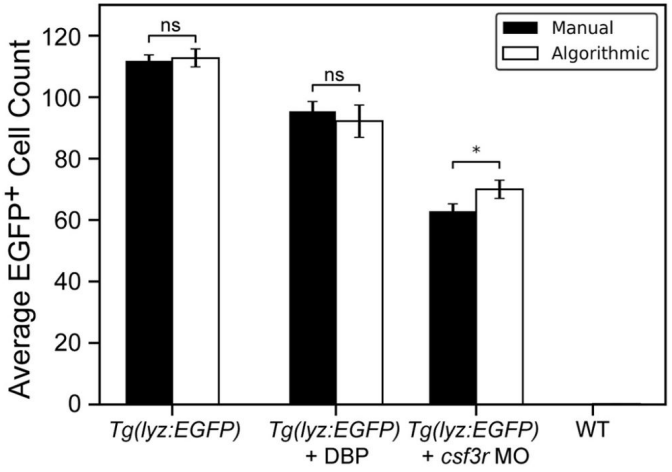

A.
B.
17.5 mm
C.
3 mm
D.
+z
−z
1 mm
E.
0.4 mm
F.
0.4 mm
G.
0.4 mm

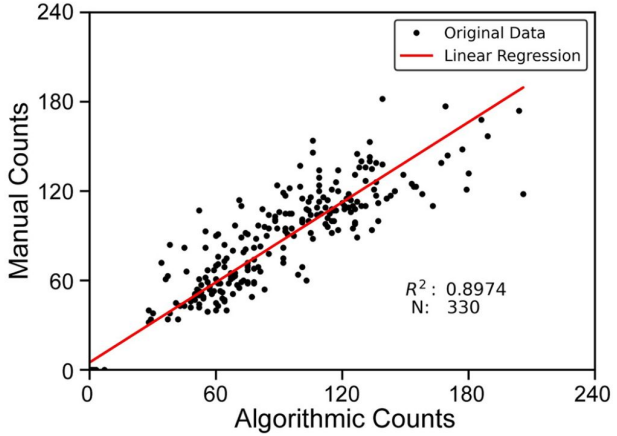

Tg(lyz:EGFP) EGFP+ Cell Count
200
150
100
50
0
N = 93
Manual
Algorithmic

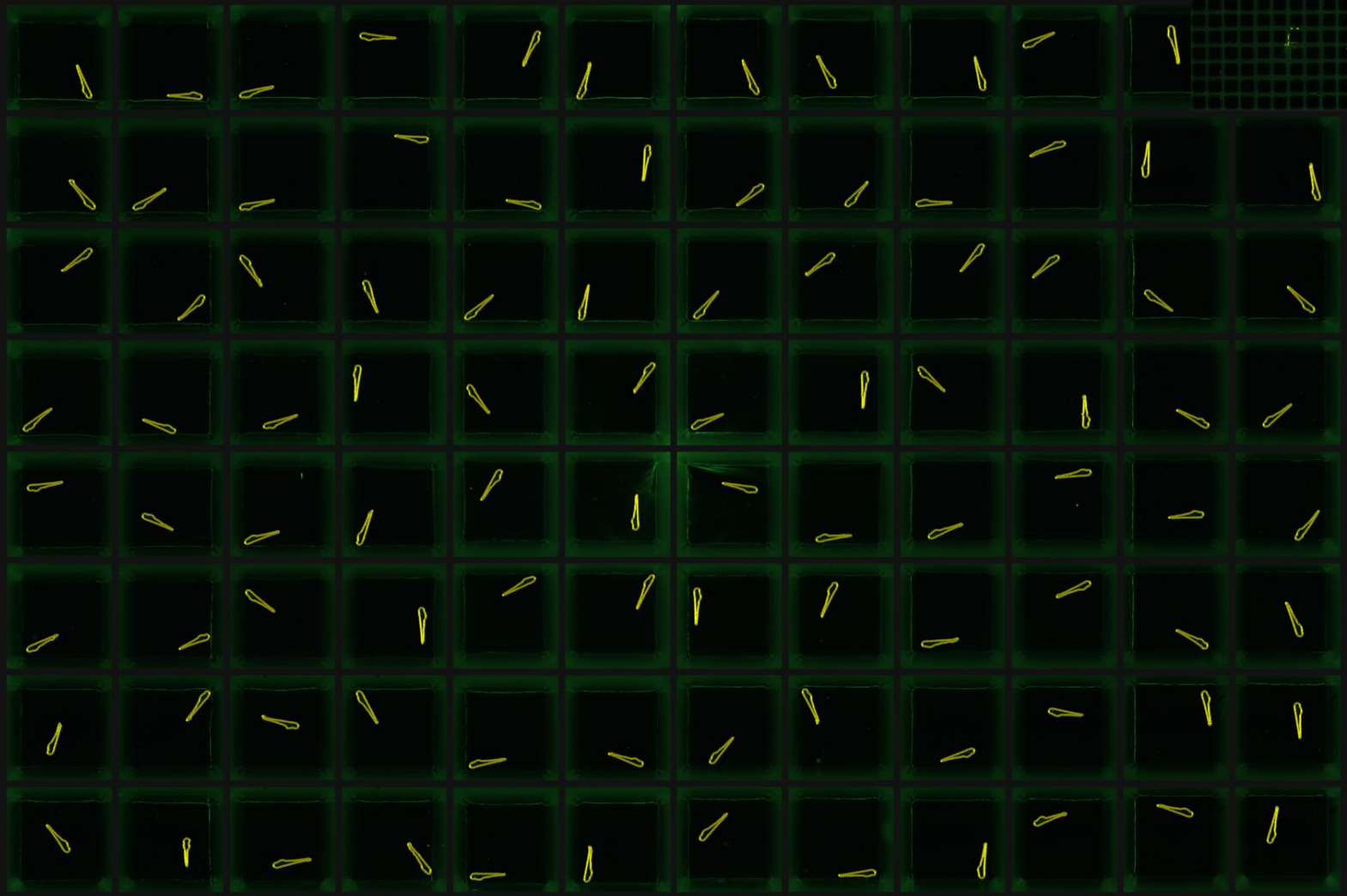

A.
B.
17.5 mm
C.
3 mm
D.
+z
−z
1 mm
E.
0.4 mm
F.
0.4 mm
G.
0.4 mm


Average EGFP+ Cell Count
120
100
80
60
40
20
0
ns
ns
*
Manual
Algorithmic
Tg(lyz:EGFP)
Tg(lyz:EGFP) + DBP
Tg(lyz:EGFP) + csf3r MO
WT


A.
B.
17.5 mm
C.
3 mm
D.
+z
−z
1 mm
E.
0.4 mm
F.
0.4 mm
G.
0.4 mm


Average EGFP+ Cell Count
120
100
80
60
40
20
0
ns
ns
*
Manual
Algorithmic
Tg(lyz:EGFP)
Tg(lyz:EGFP) + DBP
Tg(lyz:EGFP) + csf3r MO
WT


Manual Counts
240
180
120
60
0
Original Data
Linear Regression
R^2: 0.8974
N: 330
0
60
120
180
240
Algorithmic Counts



Segmentation

Segmentation Analysis Protocol

Load | Save

Model Selection

Zebrafish Neutrophils (Version 20240314)

Load Custom Model

Batch Analysis

Choose Default Settings

Segmentation Options

Filter Regions

Analysis Options

Compute Region Areas

Units: Millimeters

Pixel Intensity Threshold: 55

Color Channel: Green

Compute Fluorescence Intensity

Count Blobs

Export Settings

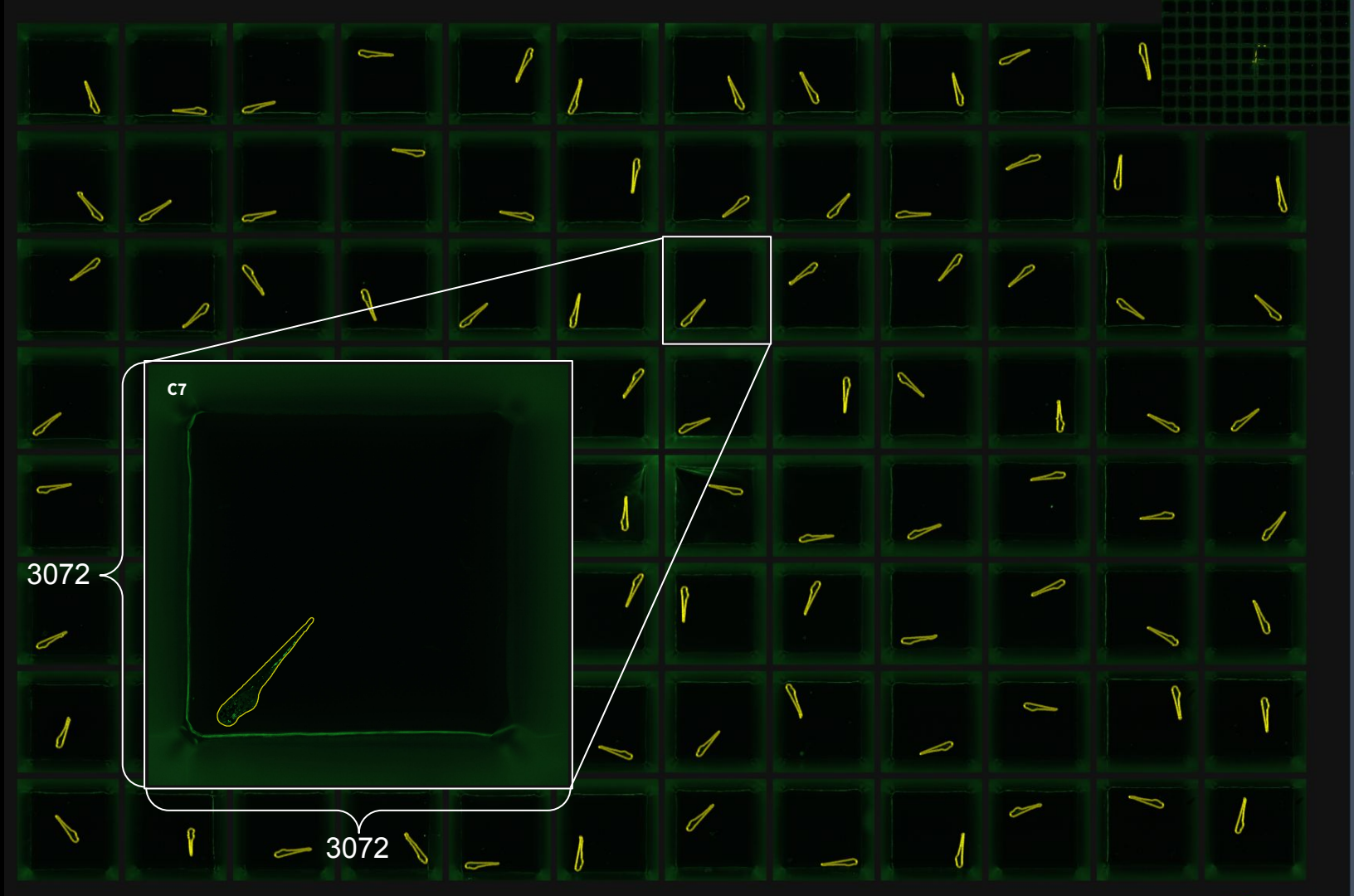
Combine CSV Results

Export Masked Images

Export Regions

Export Annotations

Segment Instances



Segmentation

Segmentation Analysis Protocol

Load | Save

Model Selection

Zebrafish Neutrophils (Version 20240314)

Load Custom Model

Batch Analysis

Choose Default Settings

Segmentation Options

- [x] Filter Regions

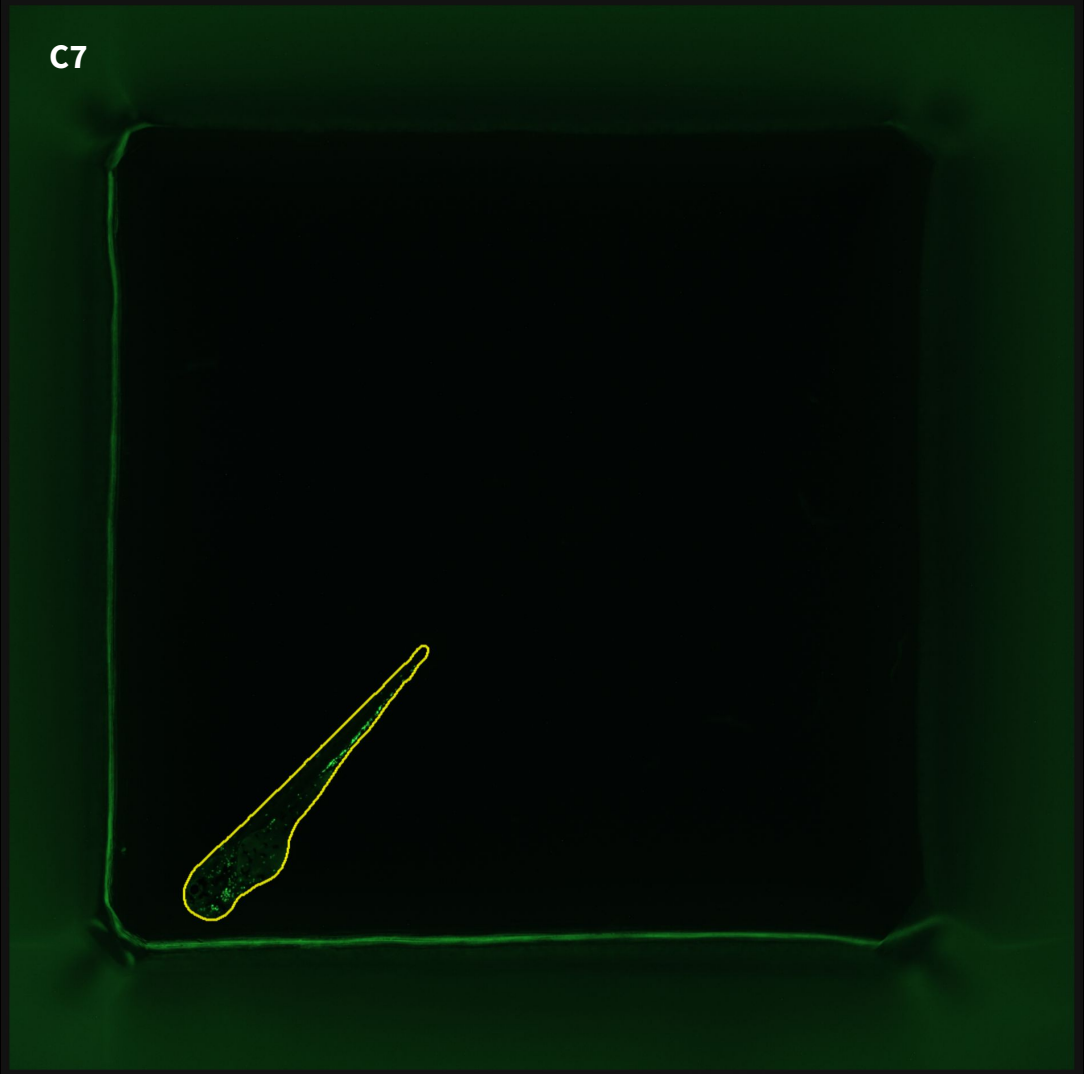
Analysis Options

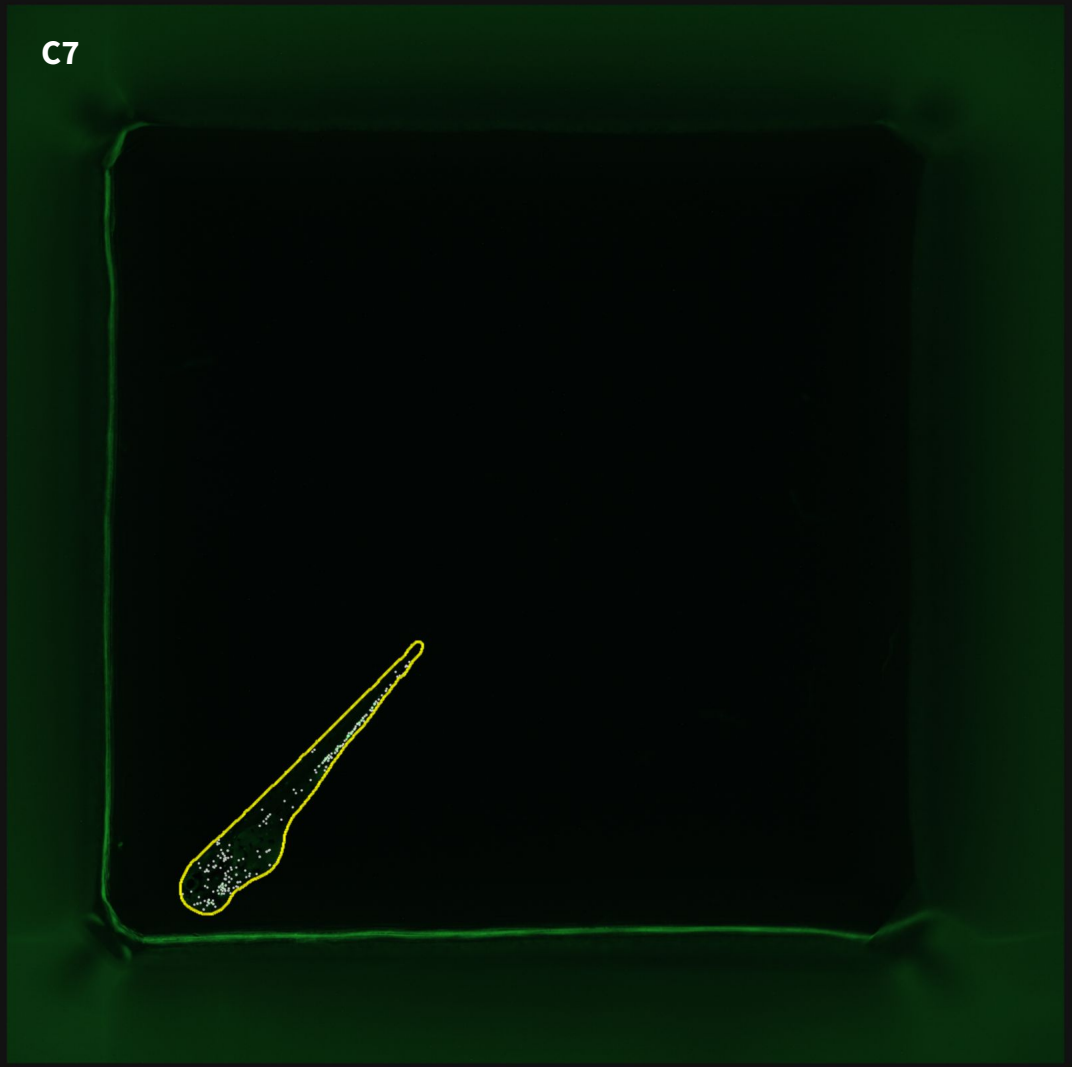
- [ ] Compute Region Areas
- Units: Millimeters
- Pixel Intensity Threshold: 55
- Color Channel: Green
- [ ] Compute Fluorescence Intensity
- [ ] **Count Blobs**

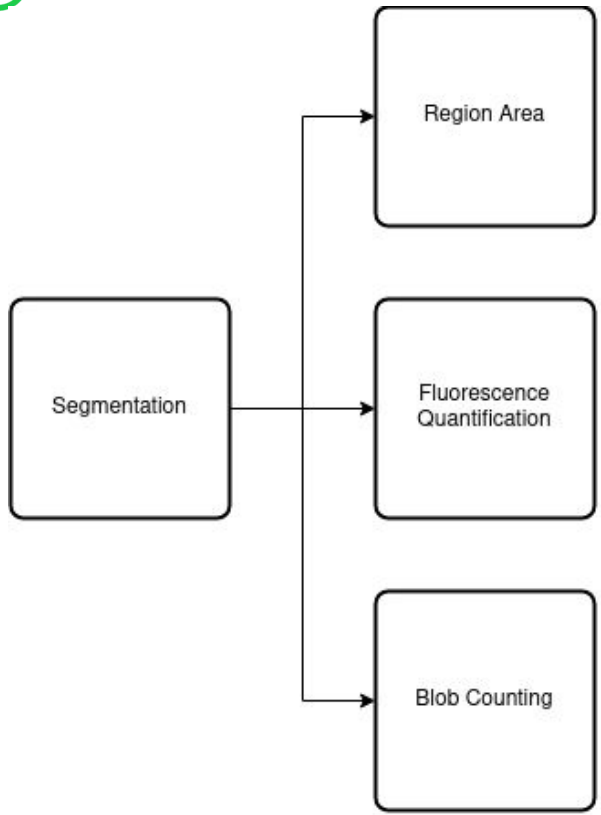
Export Settings

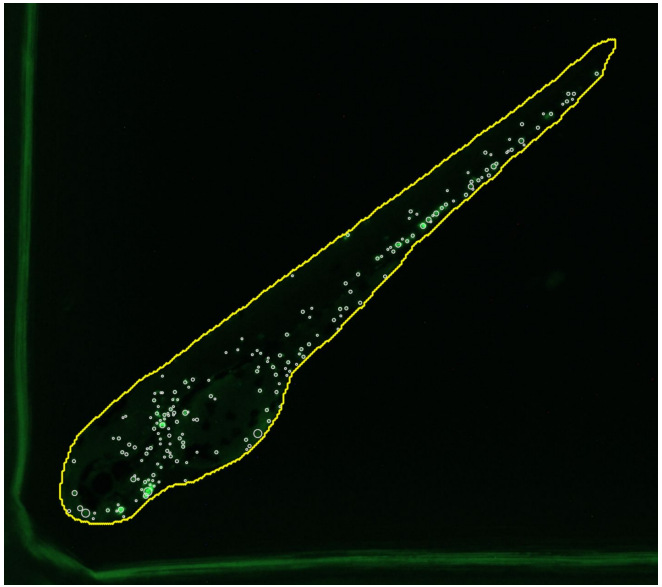
- [x] Combine CSV Results
- [ ] Export Masked Images
- [ ] Export Regions
- [ ] Export Annotations

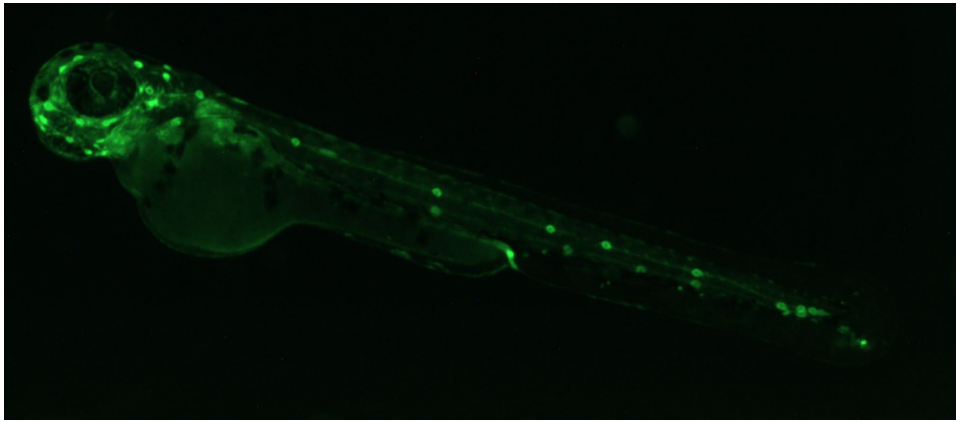
Segment Instances


C7

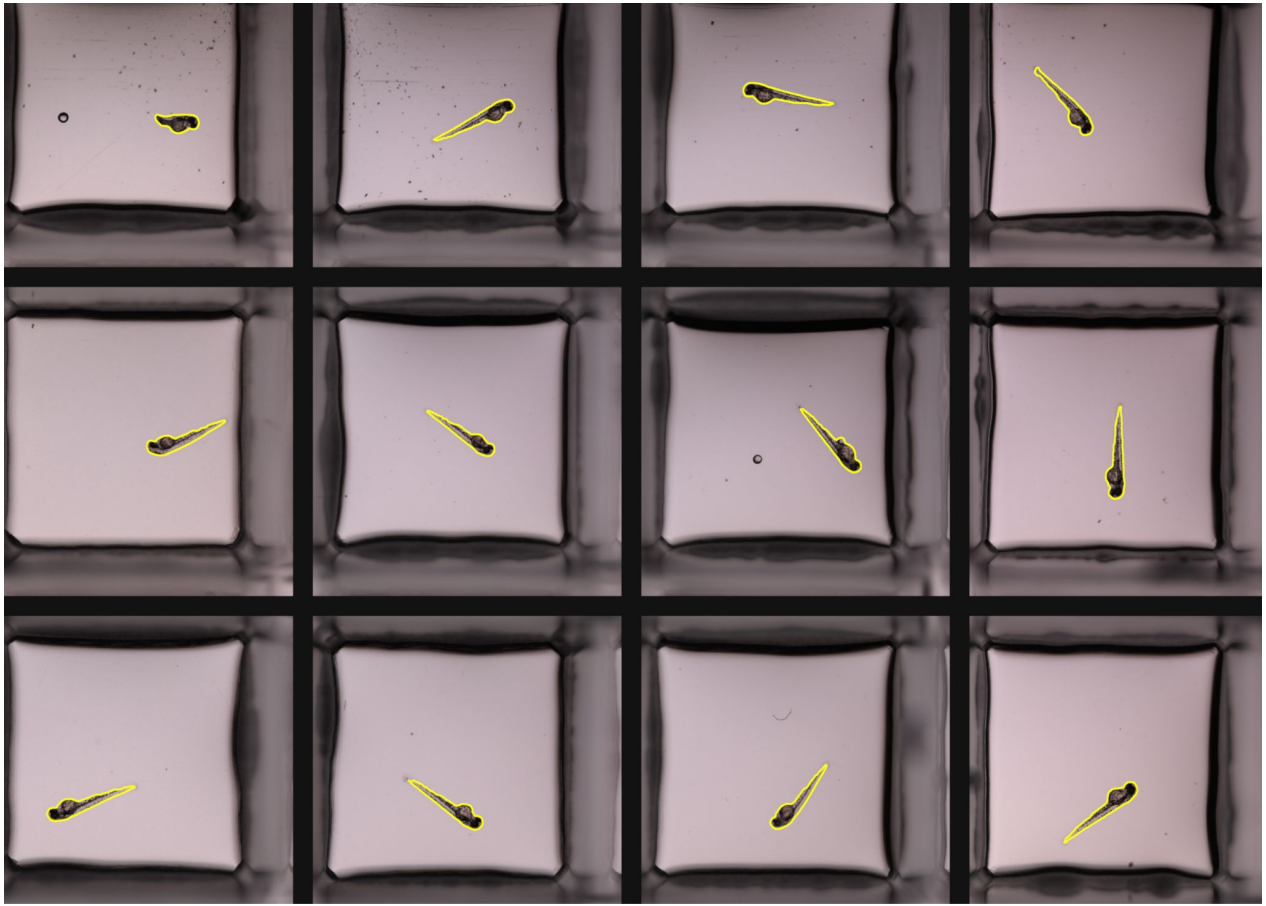

C7

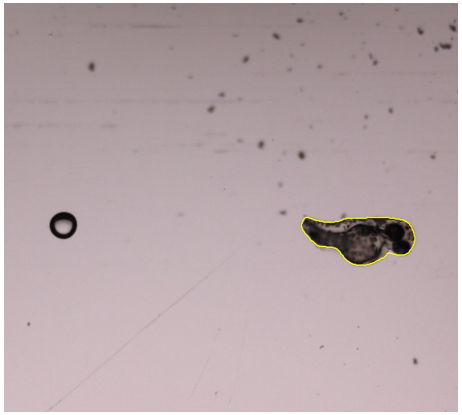

Region Area
Segmentation
Fluorescence Quantification
Blob Counting

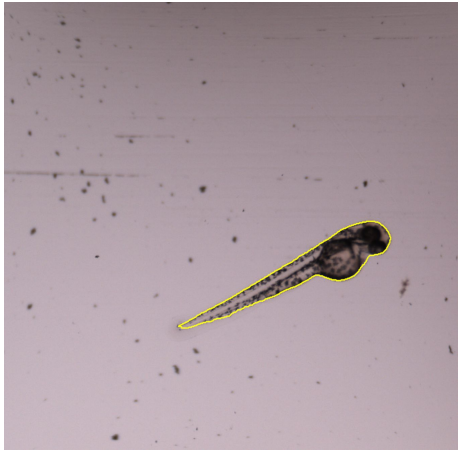




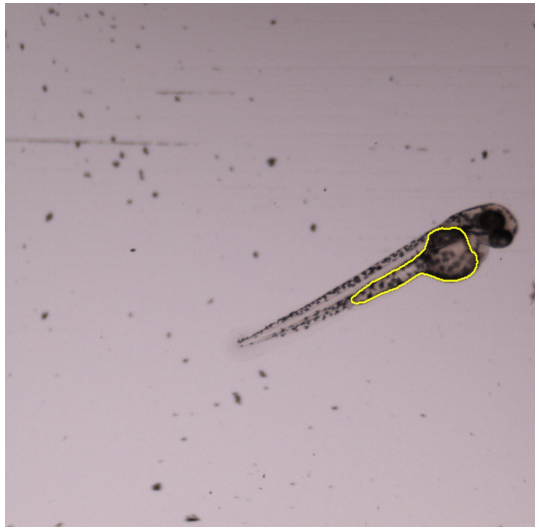
Morphology | **72 hpf Fish Area**



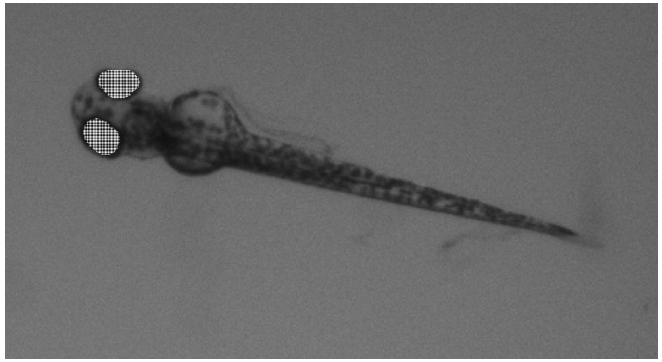


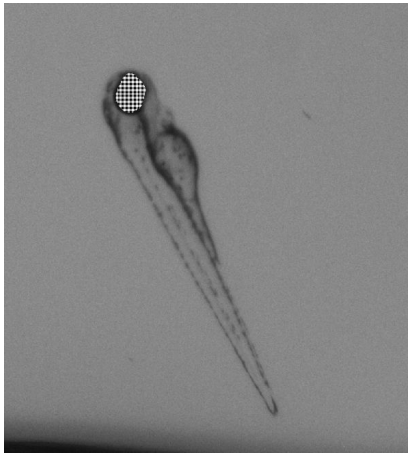


Morphology | **72 hpf Yolk Area**

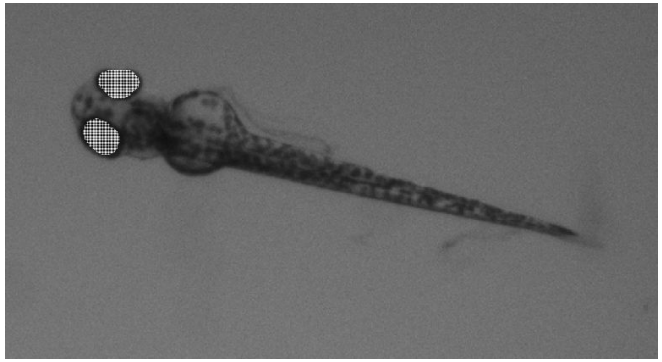


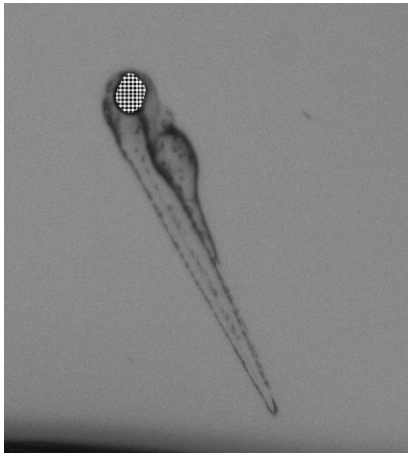
Morphology | **Eye Area**

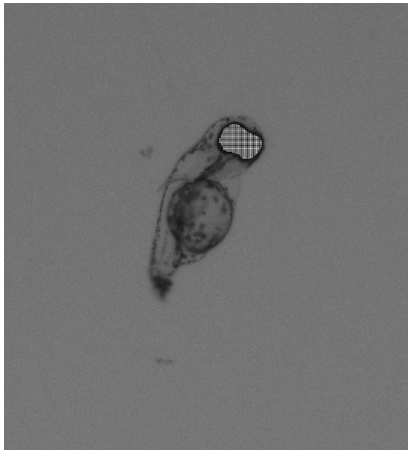




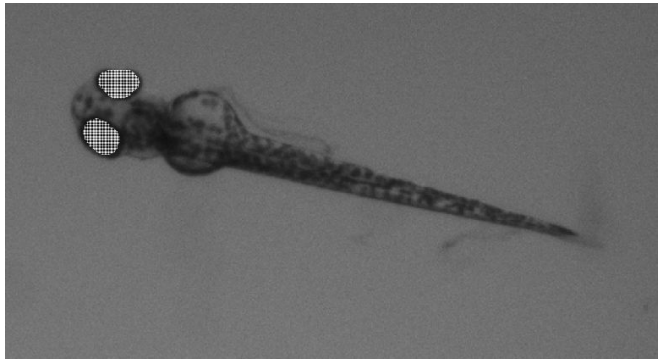
Morphology | **Eye Area**

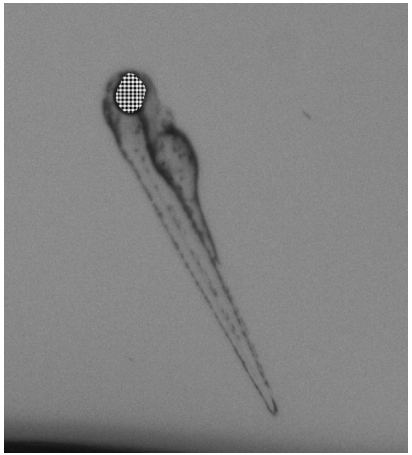


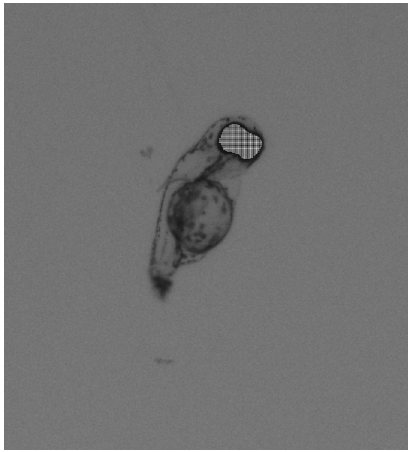




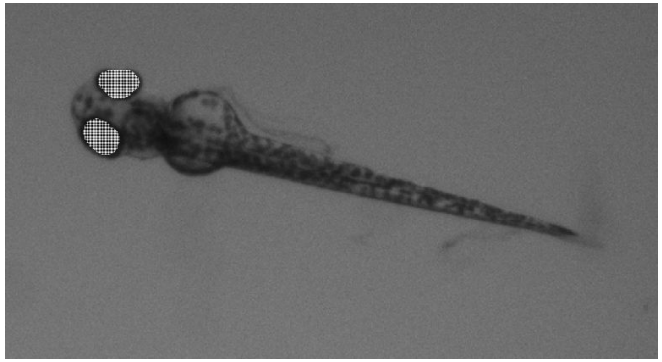
Morphology | **Eye Area**

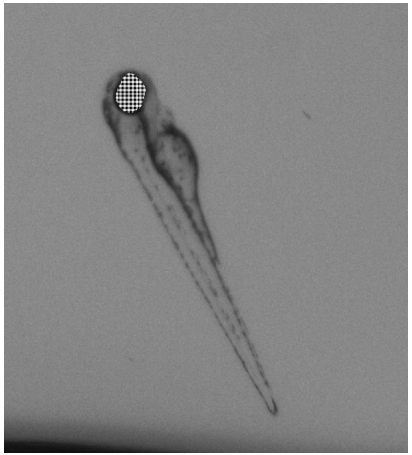


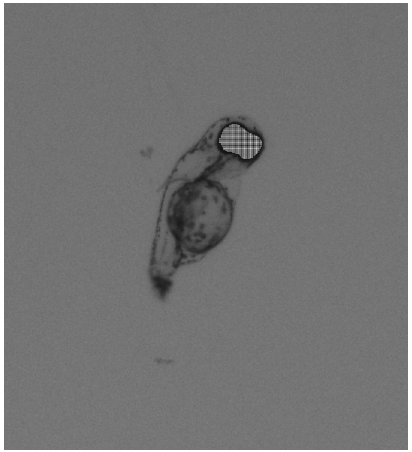


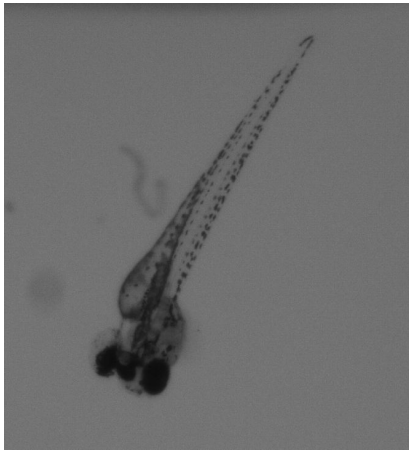


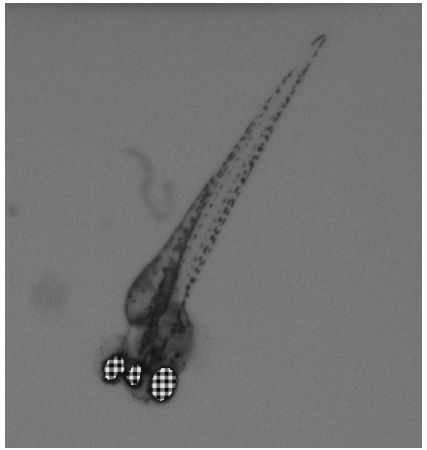
Morphology | **Eye Area**





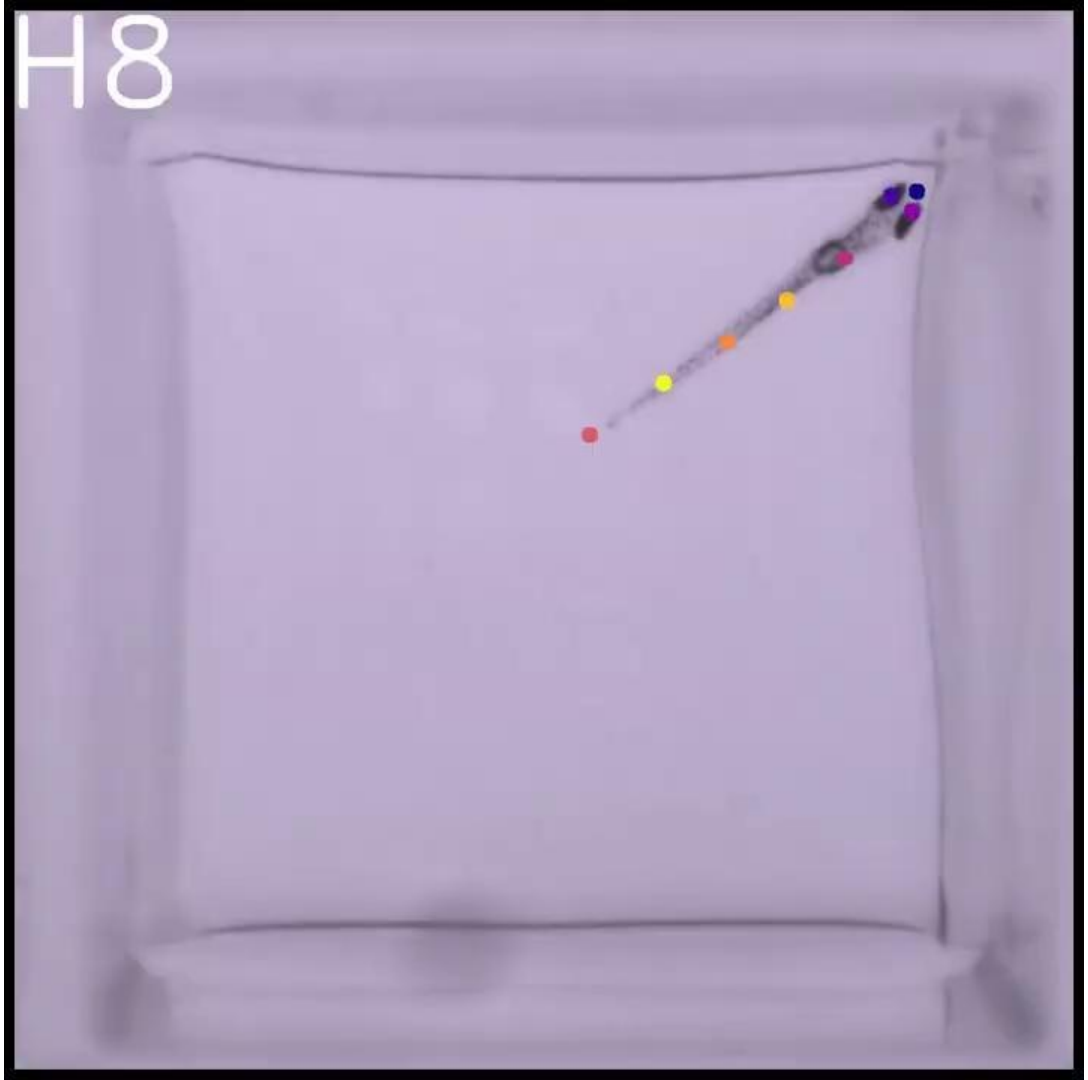






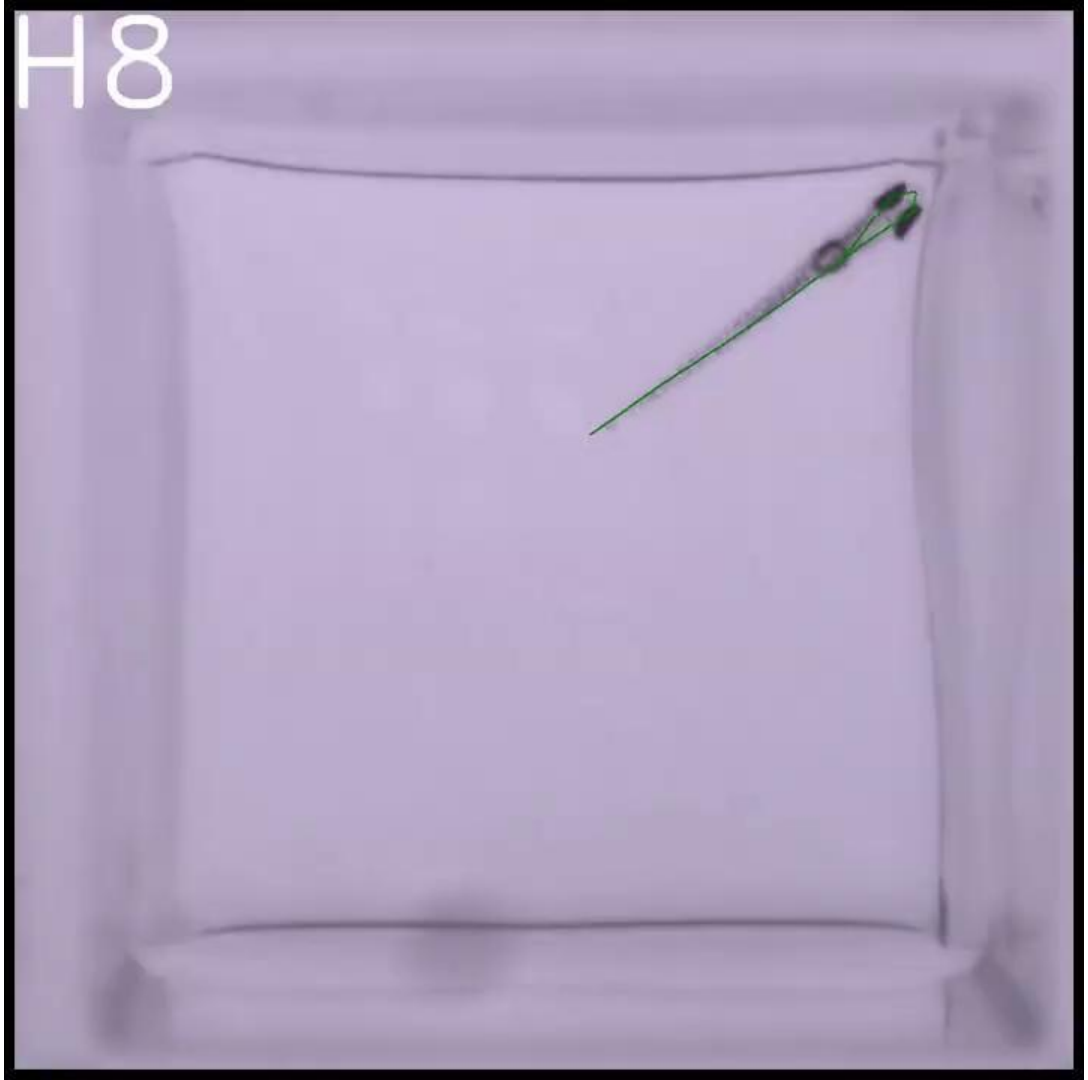
Behavior

8 Keypoint Pose Estimation



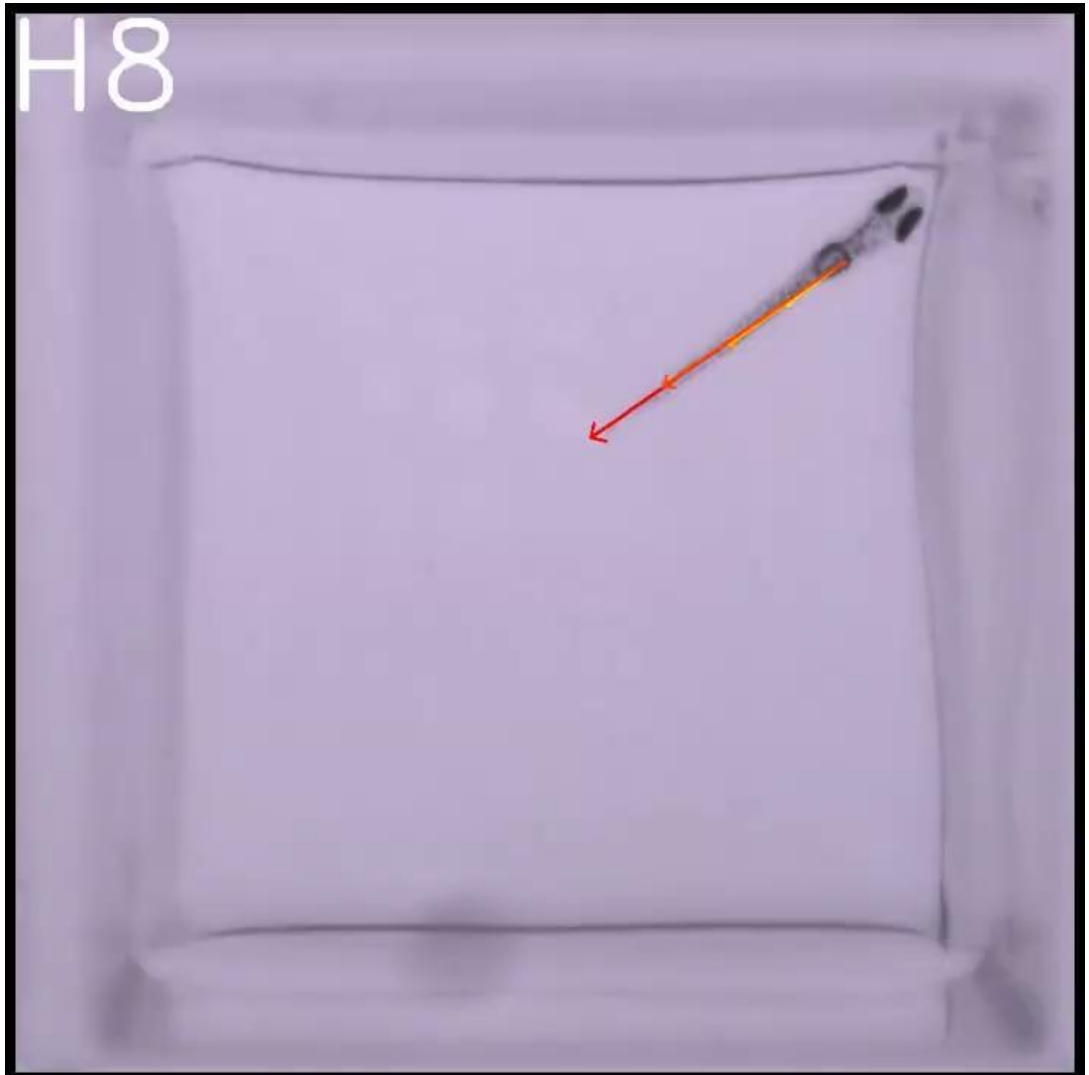
Behavior

8 Keypoint Pose Estimation

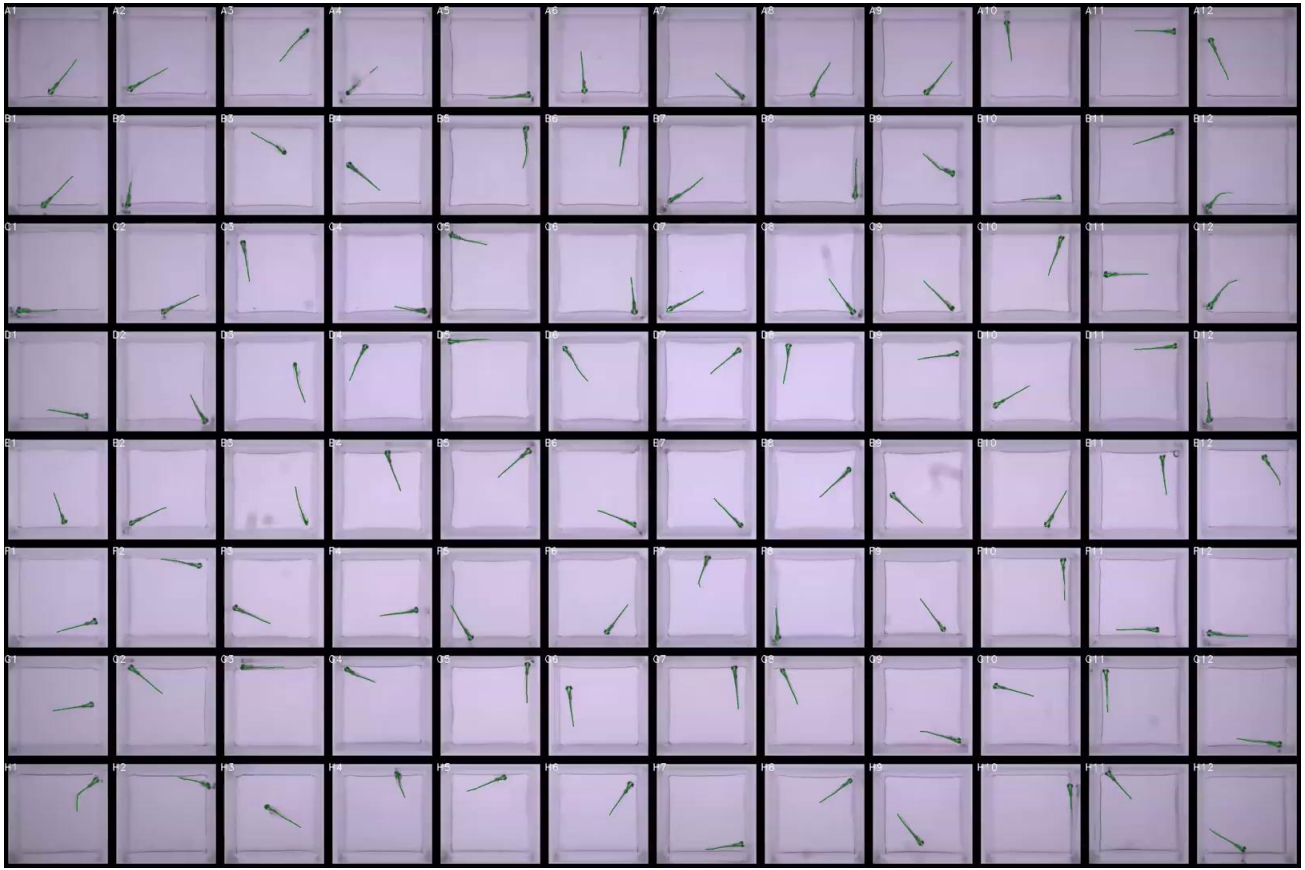


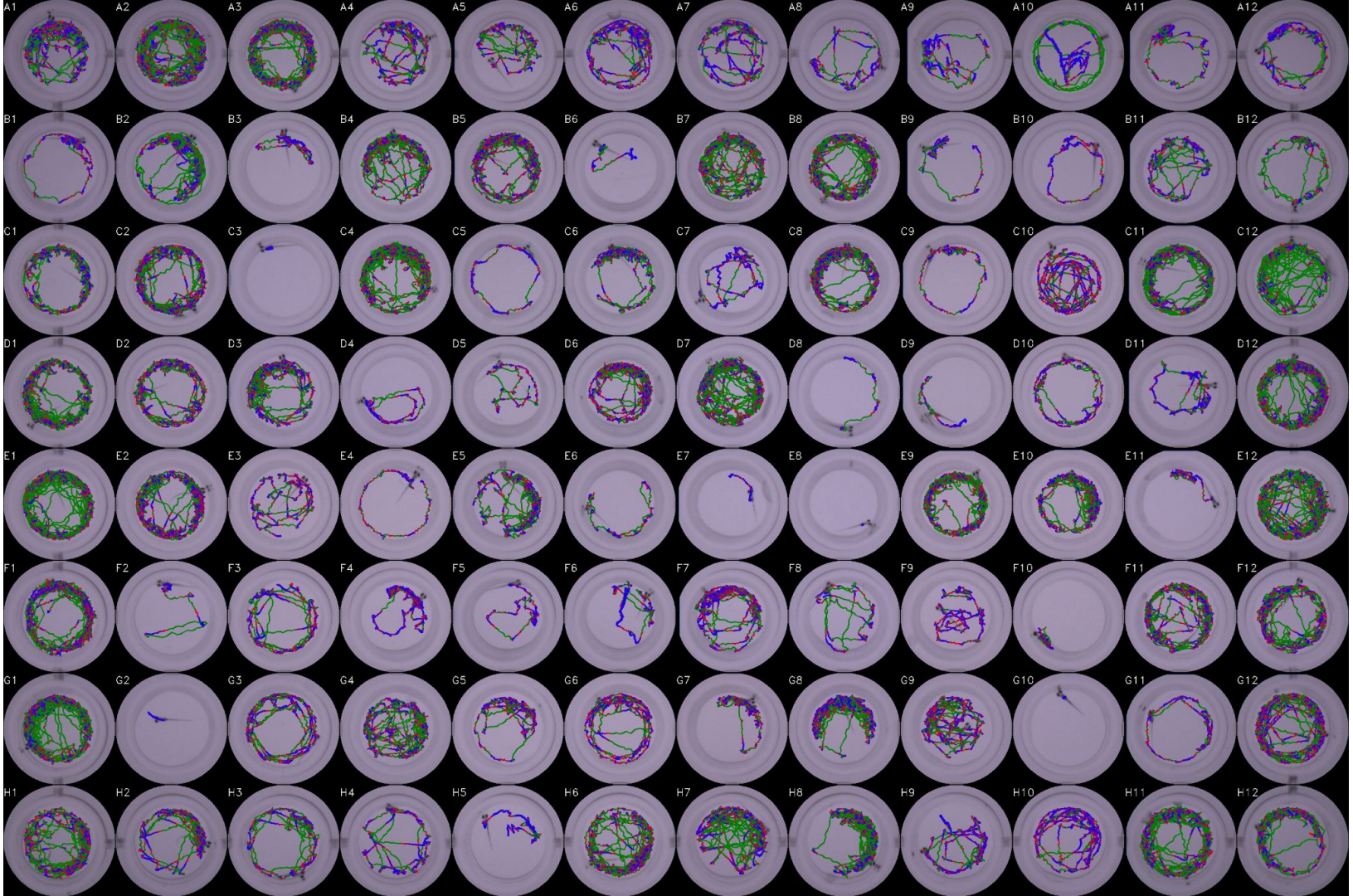
Behavior

8 Keypoint Pose Estimation



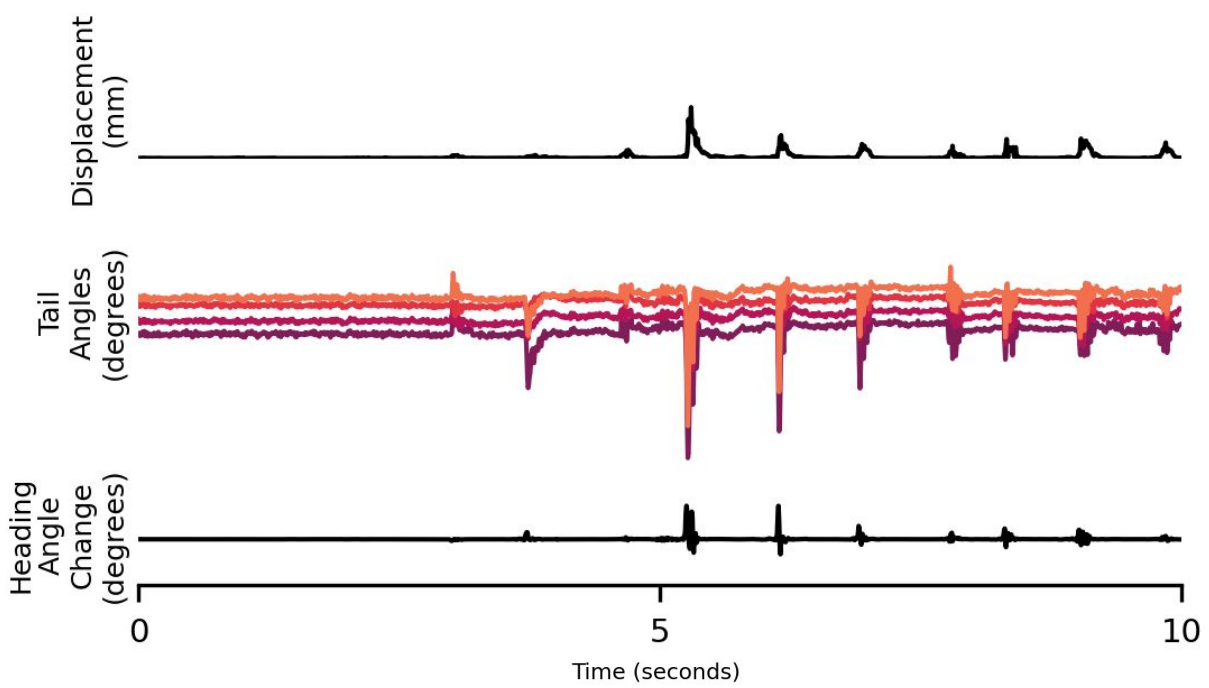
Behavior | **8 Keypoint Tracking**



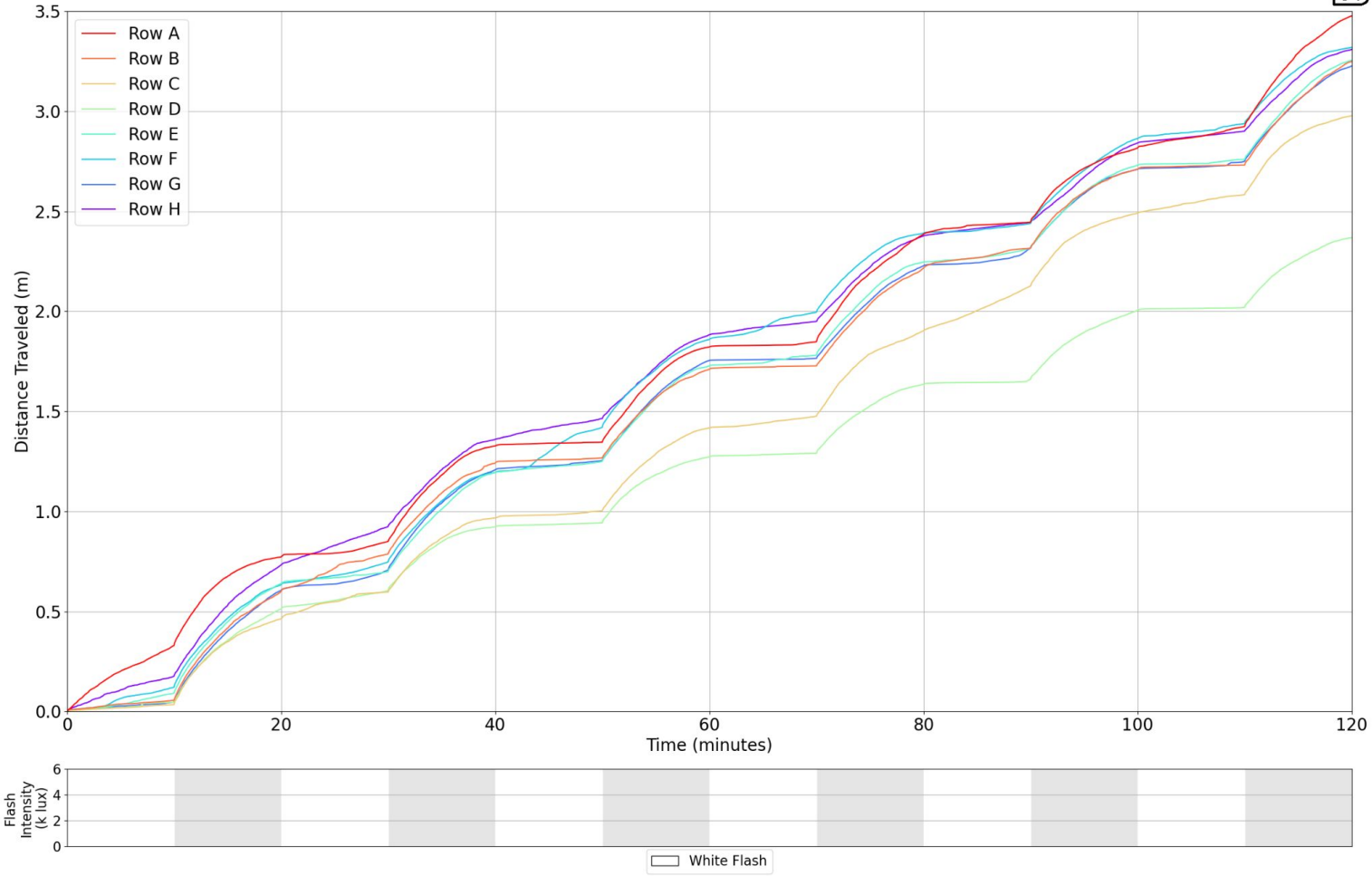

A1 A2 A3 A4 A5 A6 A7 A8 A9 A10 A11 A12
B1 B2 B3 B4 B5 B6 B7 B8 B9 B10 B11 B12
C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12
D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12
E1 E2 E3 E4 E5 E6 E7 E8 E9 E10 E11 E12
F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12
G1 G2 G3 G4 G5 G6 G7 G8 G9 G10 G11 G12
H1 H2 H3 H4 H5 H6 H7 H8 H9 H10 H11 H12

Analysis | **Synthesized Data Output**

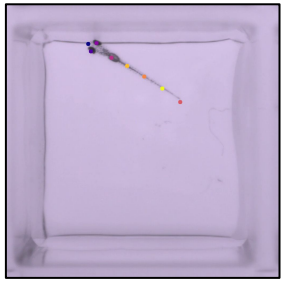
Unique behavioral statistics captured at 160 Hz across all 96 wells simultaneously

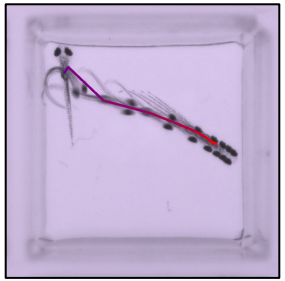


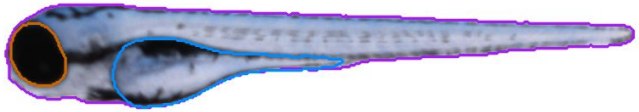
Average Distance Traveled

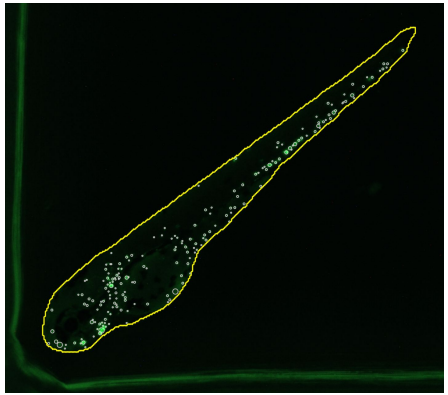


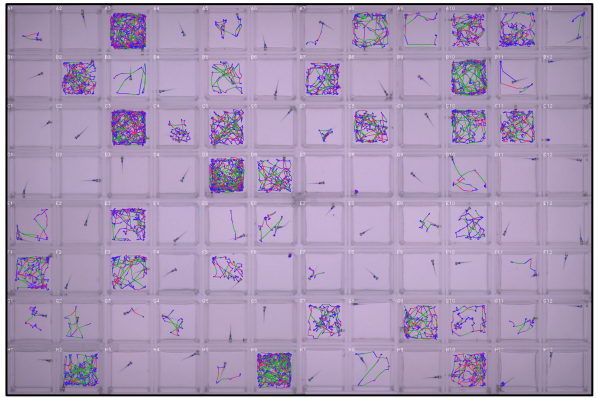
PFAS Screen - Tying Assays Together



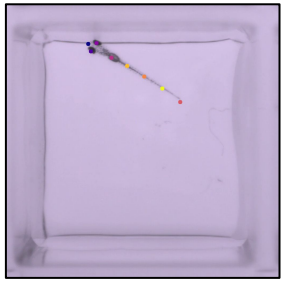


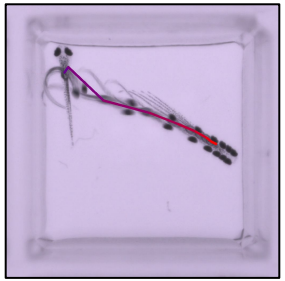


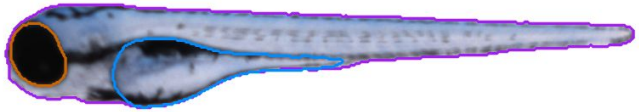


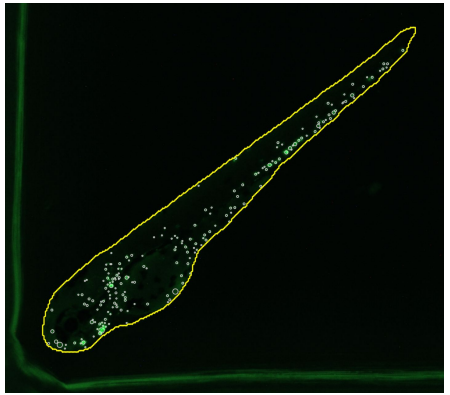


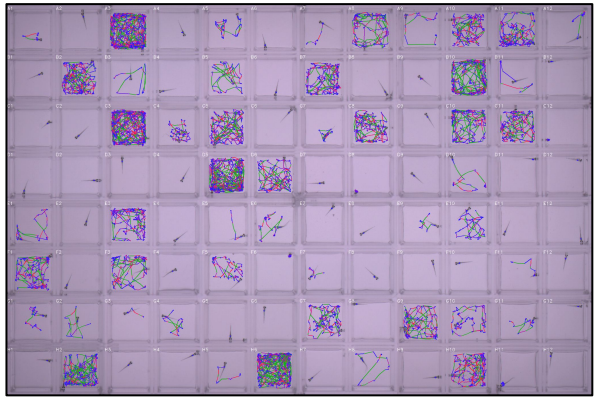
PFAS Screen - Tying Assays Together

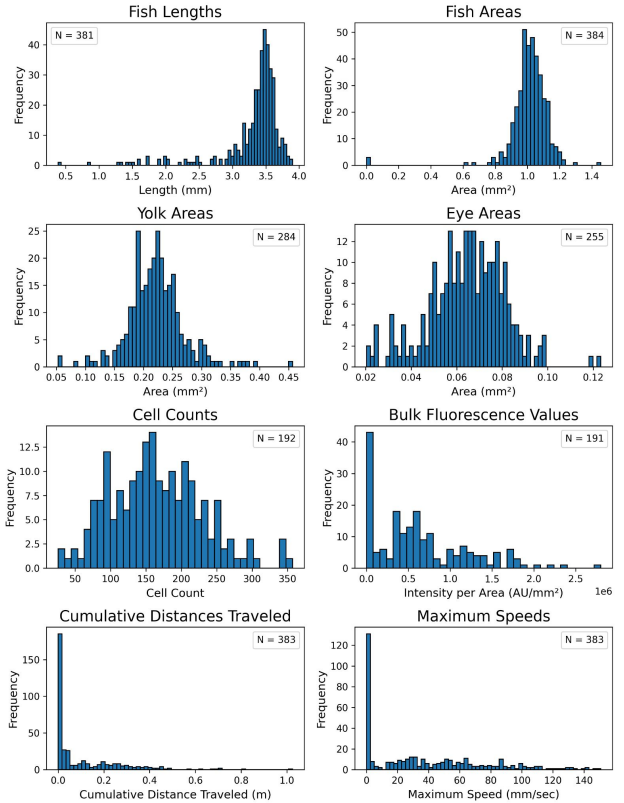




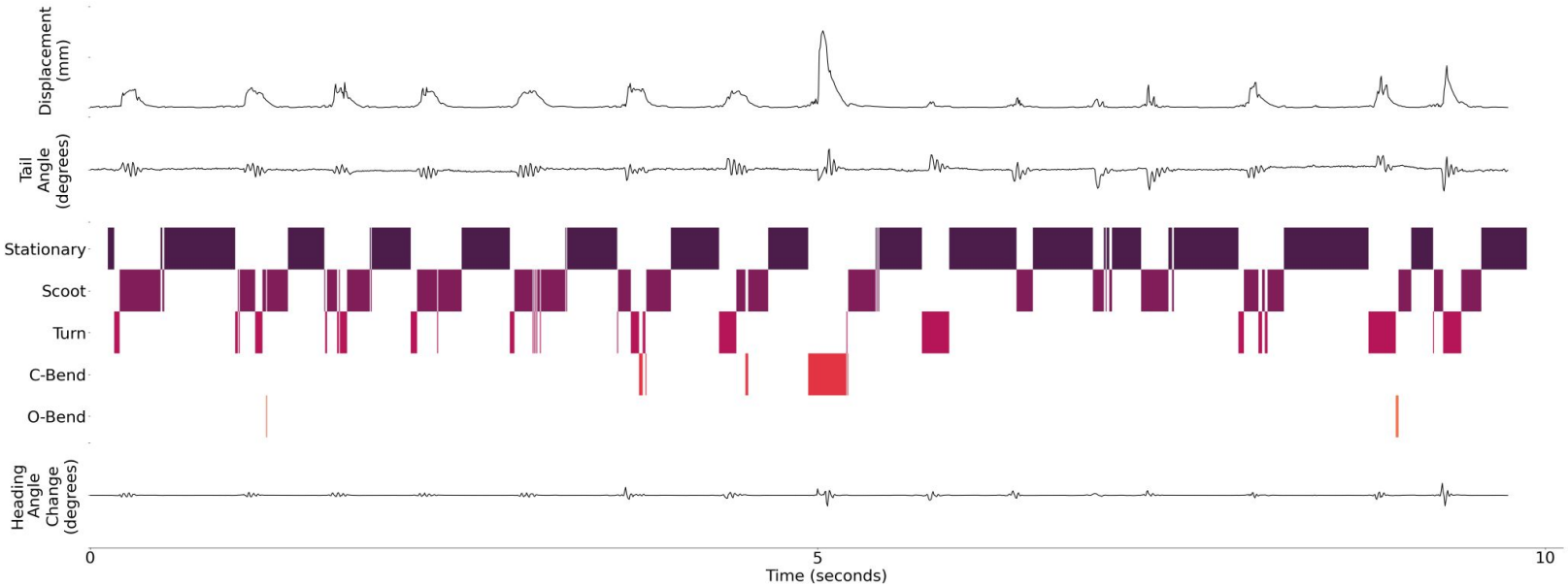








Behavior Prediction



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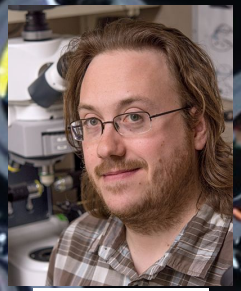

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