DeVision Project

MATH 4997

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Figure 1. A Xenopus *frog* https://aggrc.com/xenopusResearch.php

Background

Aquatic Germplasm & Genetic Resources Center (AGGRC)

- Germplasm repositories - preserve genetic diversity
- Germplasm cryopreservation for *Xenopus* frogs

Our project

- Annotation to create training data for neural network
- Develop and train a model to categorize and count embryos in new images

Figure 2. A Xenopus *frog up close*
https://aggrc.com/xenopusResearch.php

Our Project's History and Goal

History of the Project

Ongoing since Summer 2023

- Summer 2023 ➤ Counting eggs, 144 images (since decreased)
- Summer 2024 ➤ Non-viable vs Viable embryos(72 images)
- Fall 2024 ➤ VGG Image Annotation(72 images)

Goal

Utilize a StarDist-based neural network employing multiclass prediction to distinguish between stages of fertilized and unfertilized *Xenopus* frog embryos.

Manual Annotation: Workflow & Importance

1. Load embryo images into VGG Image Annotator
2. Assign a category based on division stage
3. Export the labeled data for model training

- Training a deep learning model requires labeled data.
- Poor annotation → misclassification errors.
- Manual annotation ensures model accuracy.

2. Categorizing/Annotating Embryos

- Non-split, nonviable, abnormal - **0**
- Split once (two cells) - **1**
- Split twice (four cells) - **2**
- Split three times (eight cells) - **3**
- Reflect the NF stages 2, 3, 4 (Nieuwkoop and Faber)

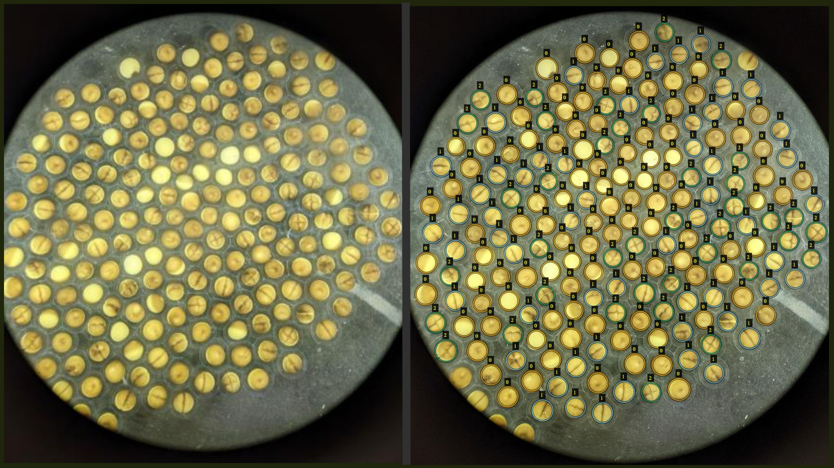


Figure 4. Image of Xenopus laevis *embryos before and after annotation*

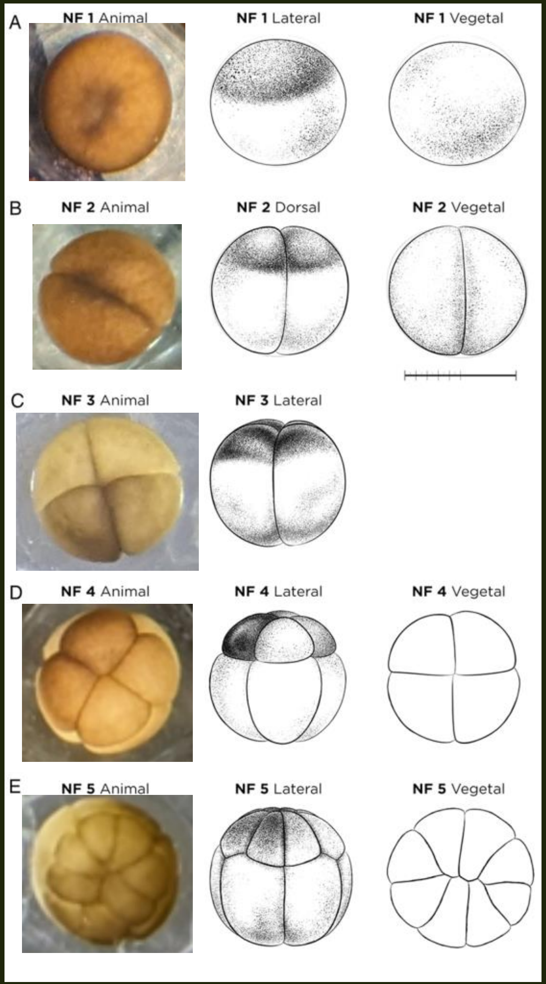
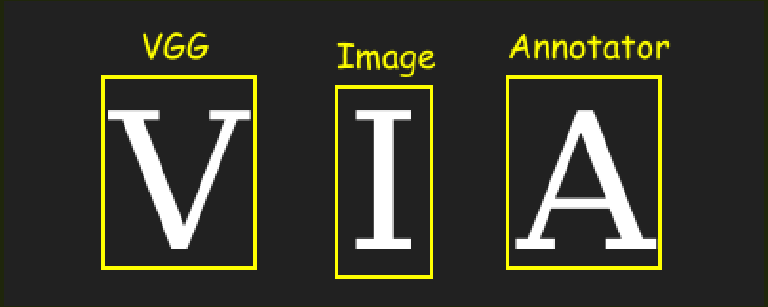


Figure 5. Zahn et al. (2022). Cleavage stages of X. laevis *embryos with examples*

1. VGG Image Annotator (VIA)

- Open source
- HTML, JavaScript, CSS
- Manual annotation software
- Runs in browser
- Uses .jpg image files



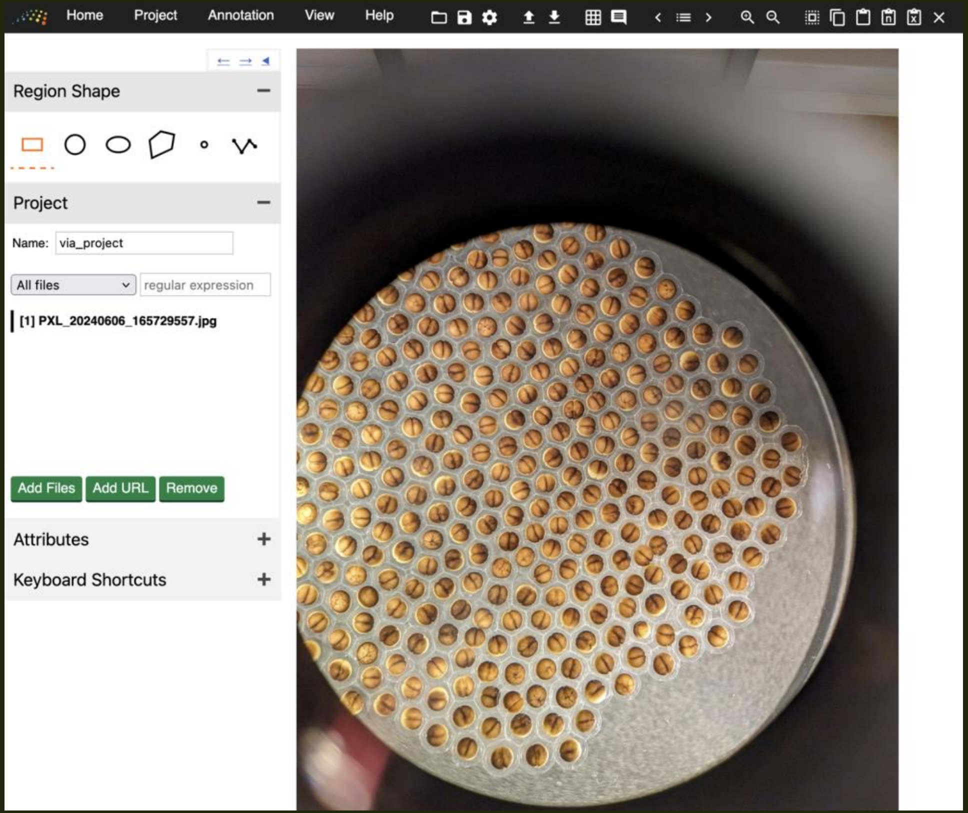


Figure 3. Image of VIA in browser

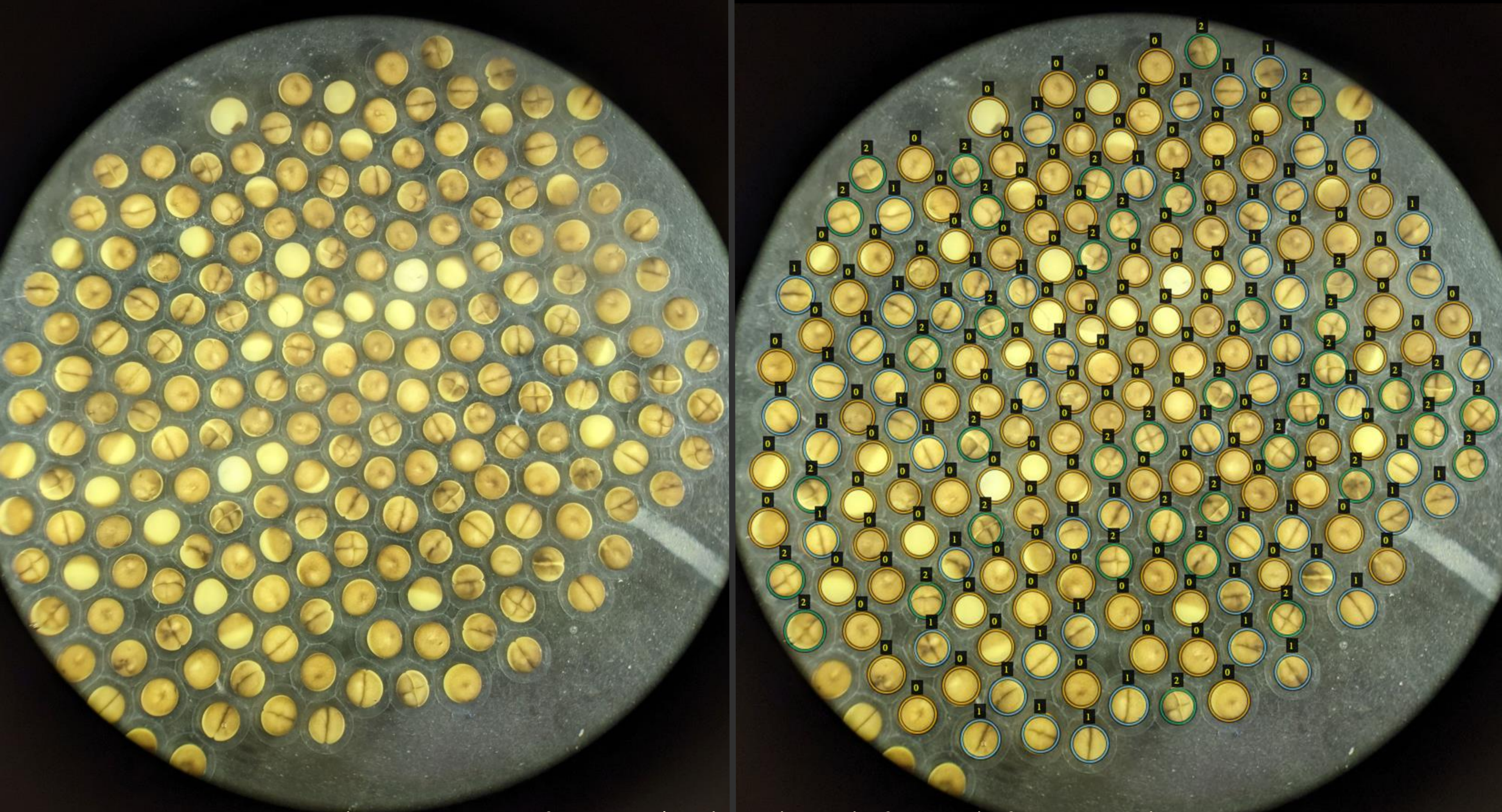


Figure 4. Image of Xenopus laevis *embryos before and after annotation*

Challenges with Image Annotations

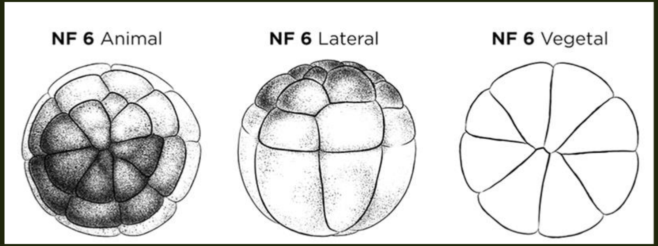


Figure 6. Zahn et al. (2022). Later cleavage stage of X. laevis *embryos*

- Time consuming
- Difficulty classifying
    - A few low-quality, blurry images
    - Rigid Categories
        - An egg with an incomplete split or split beyond 3 times may be fertilized, but these are categorized as 0s - Unfertilized/Abnormal
- 4 Category classification is more difficult than counting or binary classification
    - Model accuracy may be lower than models from previous semesters

Examples of Abnormal Embryos

Figure 7. Embryos with unusual splits and/or coloring

3. Annotation format in VGG VIA


```
ount,region_id,region_shape_attributes,region_attributes
,0,"{""name"":""circle"",""cx"":2273,""cy"":1490,""r"":67}","{""class_name"":""1""}"
,1,"{""name"":""circle"",""cx"":1228,""cy"":1233,""r"":68}","{""class_name"":""0""}"
,2,"{""name"":""circle"",""cx"":563,""cy"":1749,""r"":69.427}","{""class_name"":""2""}"
,3,"{""name"":""circle"",""cx"":605,""cy"":1924,""r"":67}","{""class_name"":""1""}"
,4,"{""name"":""circle"",""cx"":876,""cy"":1690,""r"":68}","{""class_name"":""0""}"
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,9,"{""name"":""circle"",""cx"":416,""cy"":1839,""r"":75.6}","{""class_name"":""0""}"
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,13,"{""name"":""circle"",""cx"":828,""cy"":1518,""r"":69.427}","{""class_name"":""2""}"
```


Figure 8. Snippet from the csv file for one labelled image

Translating VIA CSV files to label images

- CSV stands for **Comma Separated Values.** These files are used to store the annotation data separately from the image data.
- To make a label tensor from a csv file
  - the annotations are iteratively drawn onto an empty 1 channel image the same size as the color image, with values corresponding to the field region_id + 1
  - This value is then mapped to the corresponding class_name + 1 in the class dictionary
- Since these images are quite large, a limited number are loaded into memory at once
  - This number is known as the batch size. Larger batch sizes are able to be processed faster by utilizing the GPU, at the cost of memory and optimality.

Generated label mask

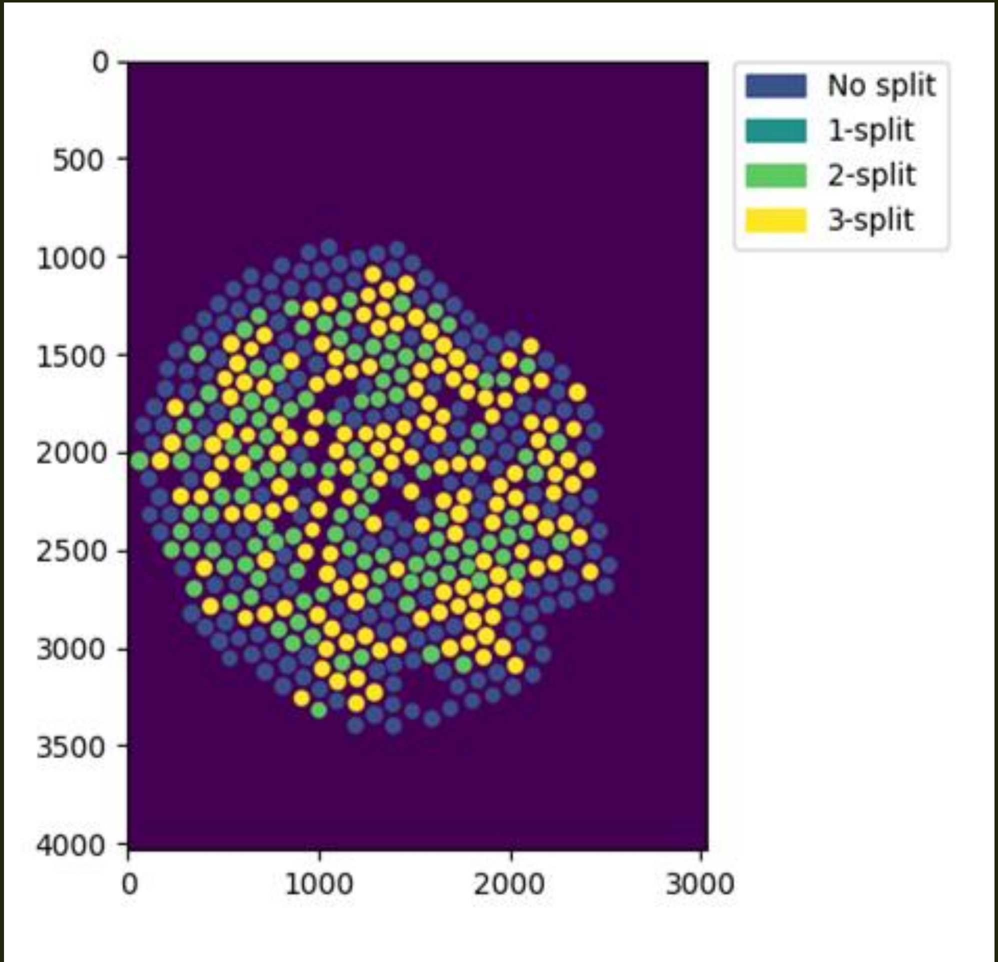

Figure 9. Label mask from one annotated image

StarDist Training Interface

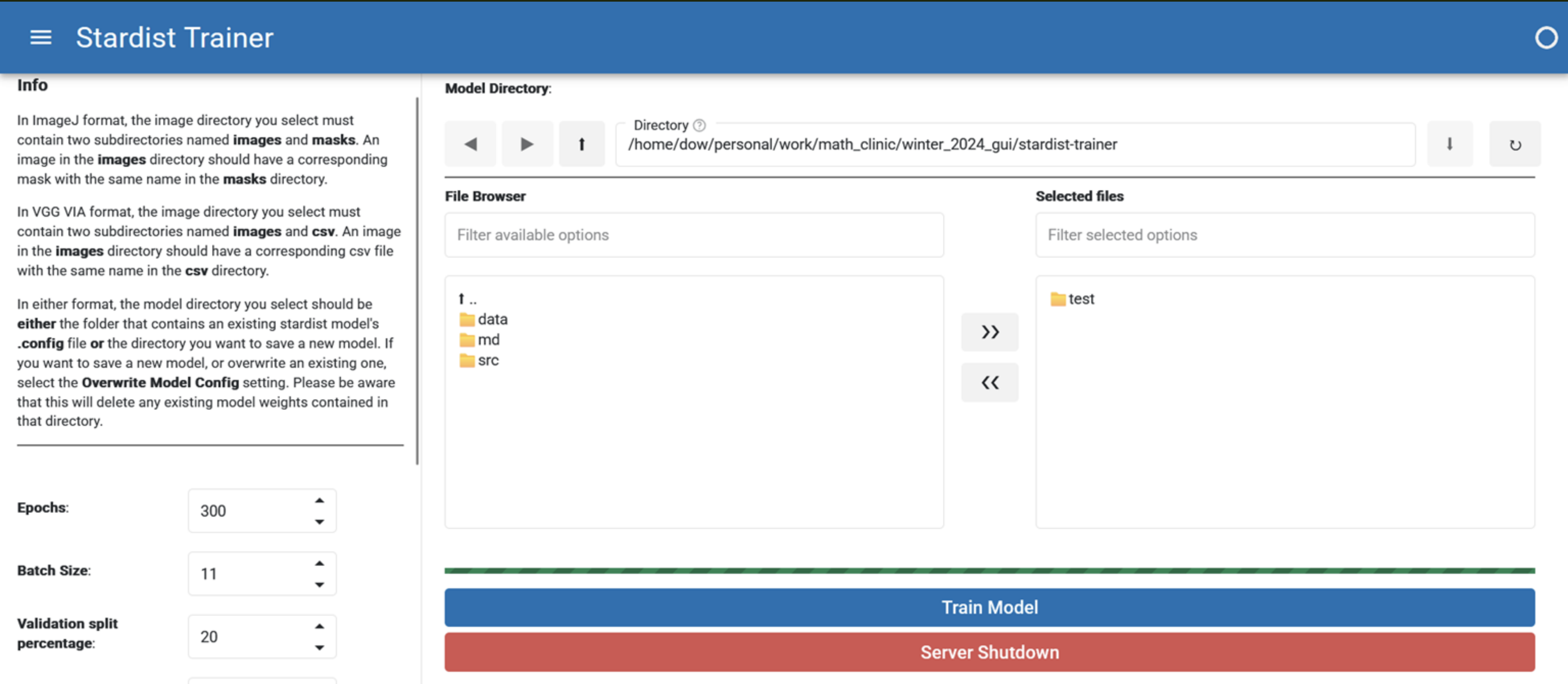

Figure 10. Screenshot of StarDist Training Interface

Flow Chart StarDist Classification Model

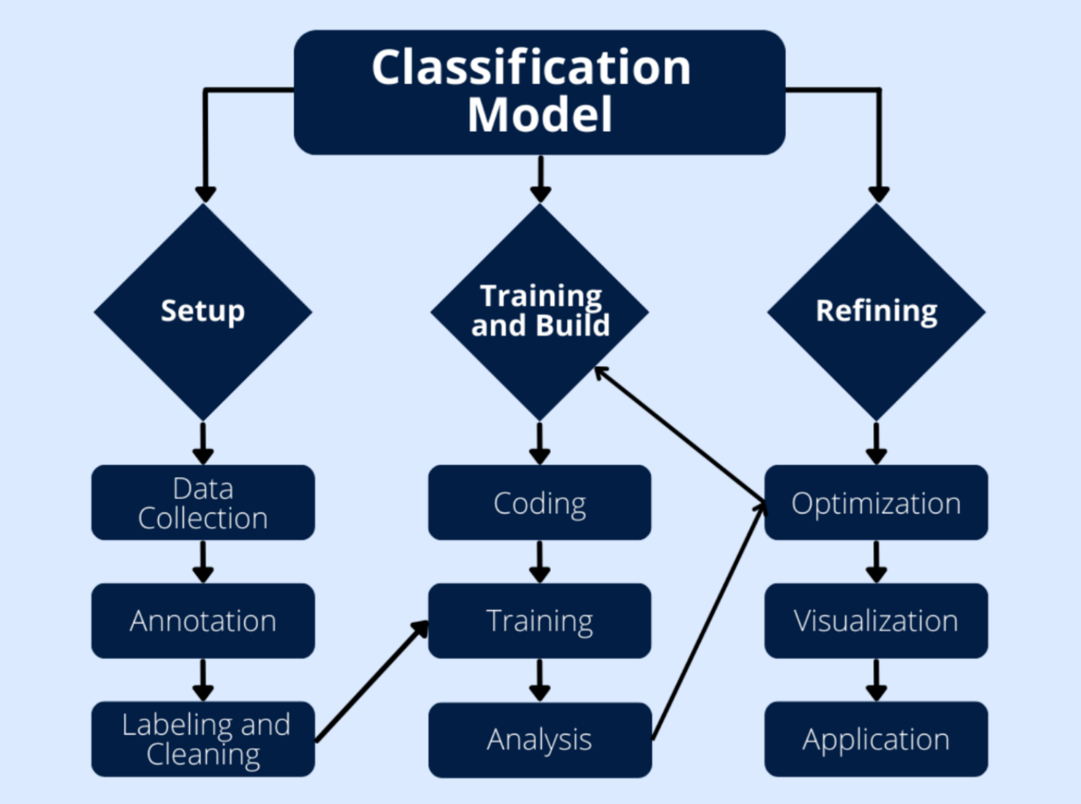

Figure 11. StarDist Classification Model Flowchart

Stardist Architecture

- U-net
  - Convolutional neural network
- Post-processing
  - IOU Metrics
  - Non-maximum suppression

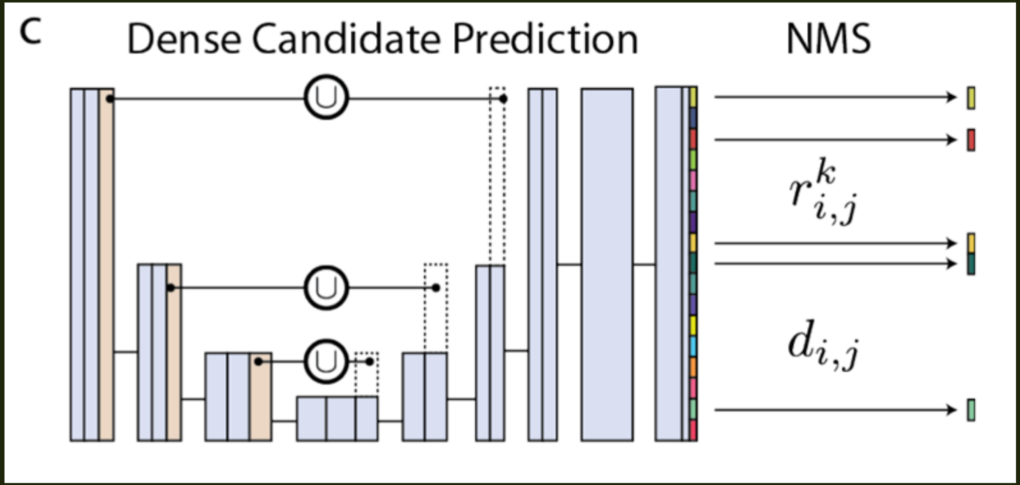

Figure 12. U-net

Stardist Architecture

- Use of star-convex polygons
- Object probabilities - how likely a pixel is a part of an object. Pixels near cell's center are favored.
- Predicts distance to object's boundaries along 32 radial distances
- Polygon candidates
- Class probabilities - aggregated to classify each object instance

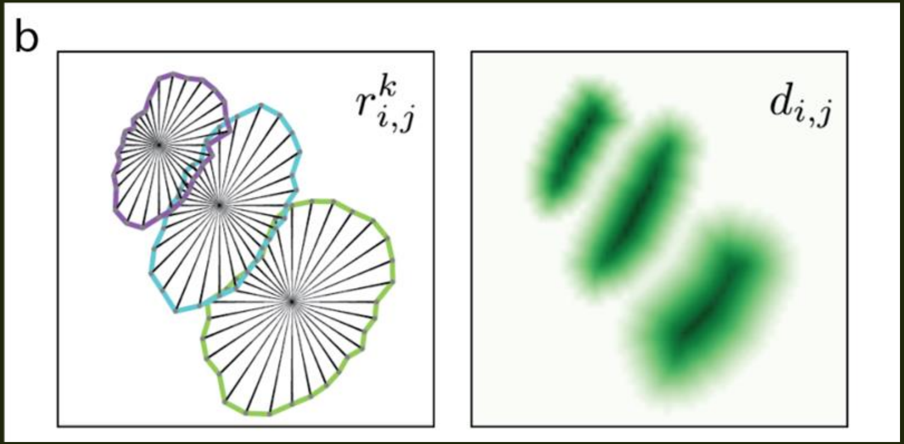

Figure 13. Star-convex distances and object probabilities

Training a multiclass annotator with StarDist

- Image data can be represented by a **tensor**, a higher dimensional matrix with the shape representing the **width * height * channels**.
- To train a multiclass StarDist model with color using the python bindings, you need three things:
  - A tensor of the input color image in the shape **w * h * 3**
  - A tensor of the label image in the shape **w * h * 1**
  - A mapping between object instances and the classes we wish to predict
- Label image values represent **object instance** values. All background pixels take value 0, all pixels belonging to the first embryo are value 1, all pixels of the second are value 2, etc.
- Each **object instance** is associated with a **class** in a surjective relation, this is given to the model as a dictionary object.

Heatmaps

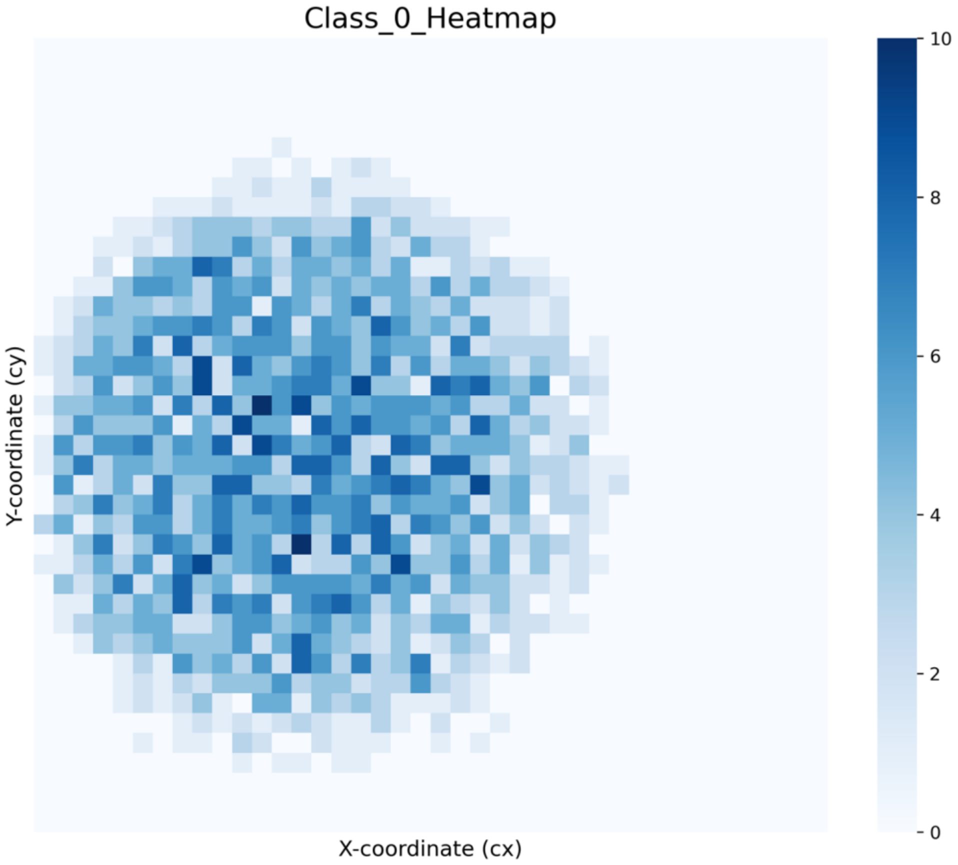

Figure 14. Density of non-split, nonviable, abnormal embryos

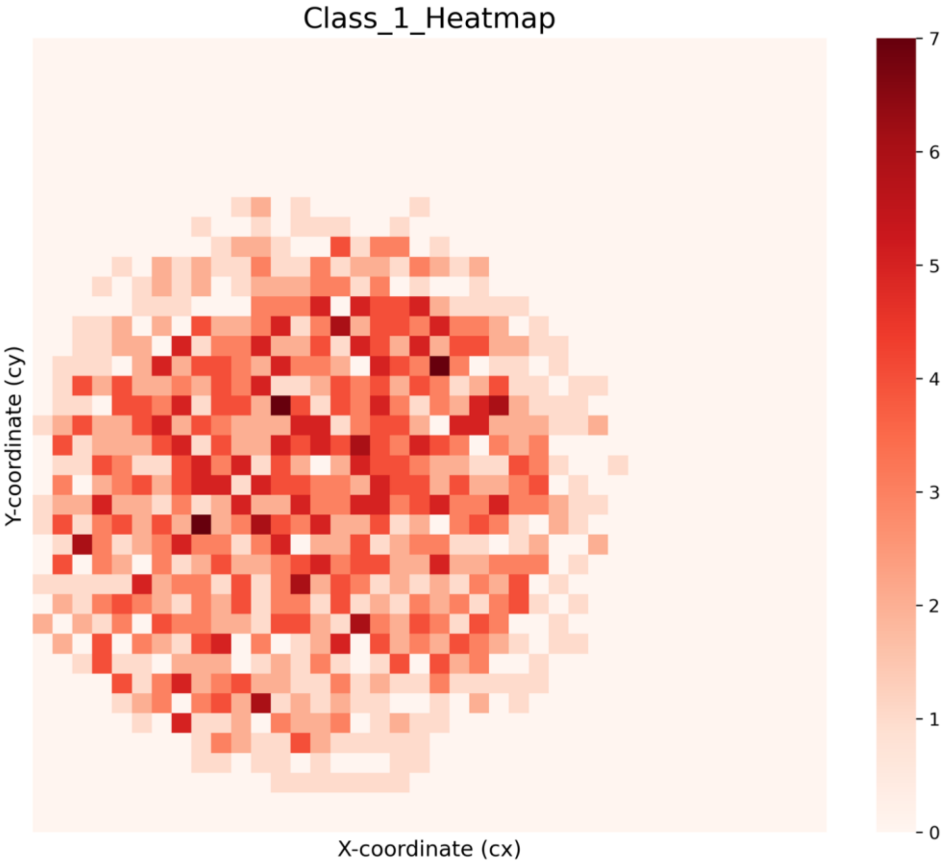

Figure 15. Density of fertilized, single-split embryos

Heatmaps

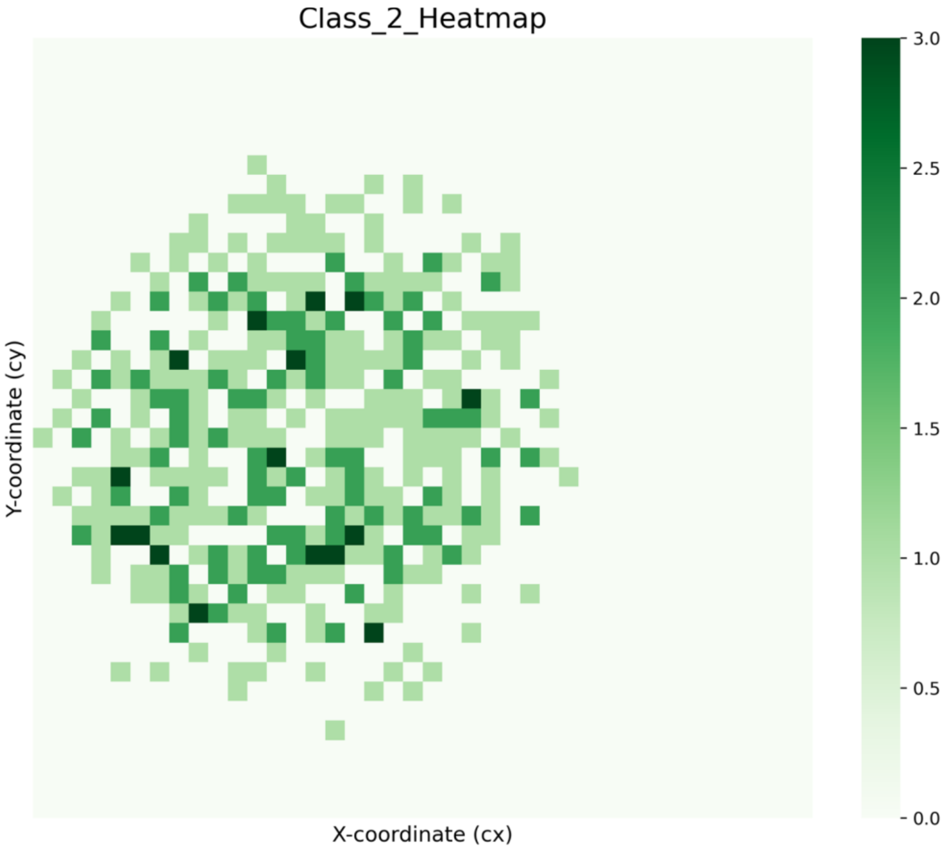

Figure 16. Density of fertilized, twice-split embryos

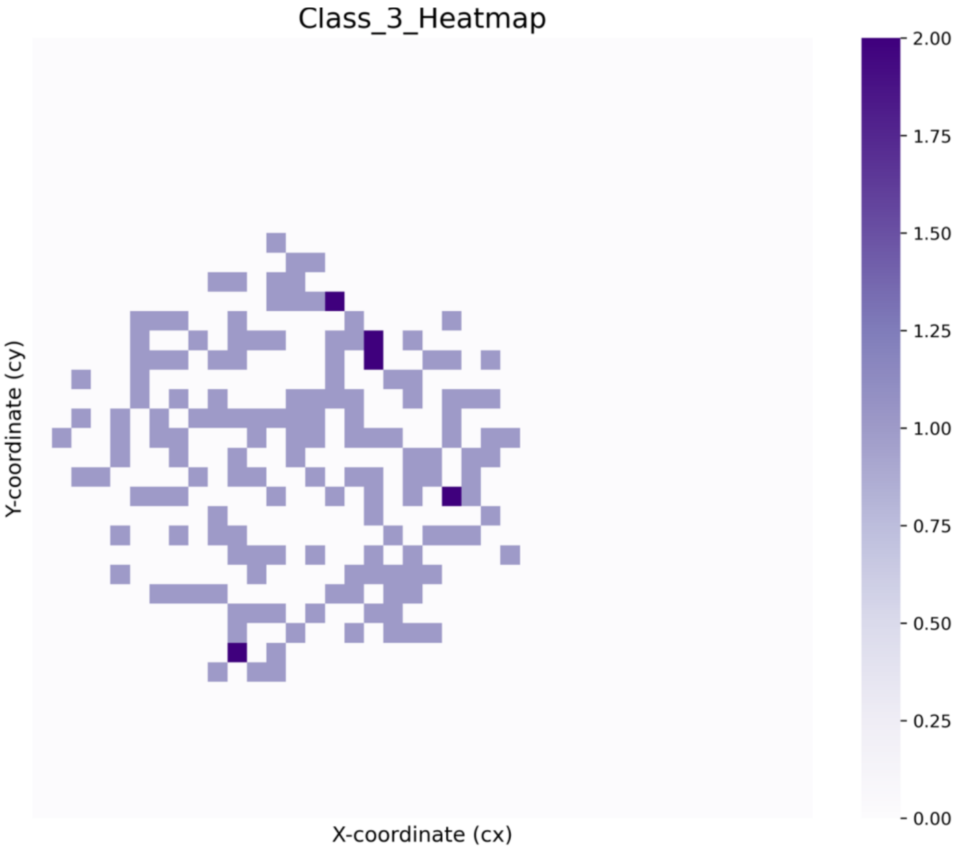

Figure 17. Density of fertilized, thrice-split embryos

Evaluation Metrics Loss Function

The loss function, a metric of the model's accuracy, being minimized:

$$L(p, p', r, r') = L_{\mathsf{prob}}(p, p') + p' L_{\mathsf{dist}}(p, p', r, r')$$

where (p, r) are the predictions and (p', r') is the ground truth.

$$L_{\mathsf{prob}}(r, r') = -r' \ln(r) - (1 - r') \log(1 - r)$$

$$L_{\mathsf{dist}}(r, r') = \frac{1}{n} \sum_{k} |r_k - r'_k|$$

Figure 18. Loss Function

Evaluation Metrics Distance and Probability Loss

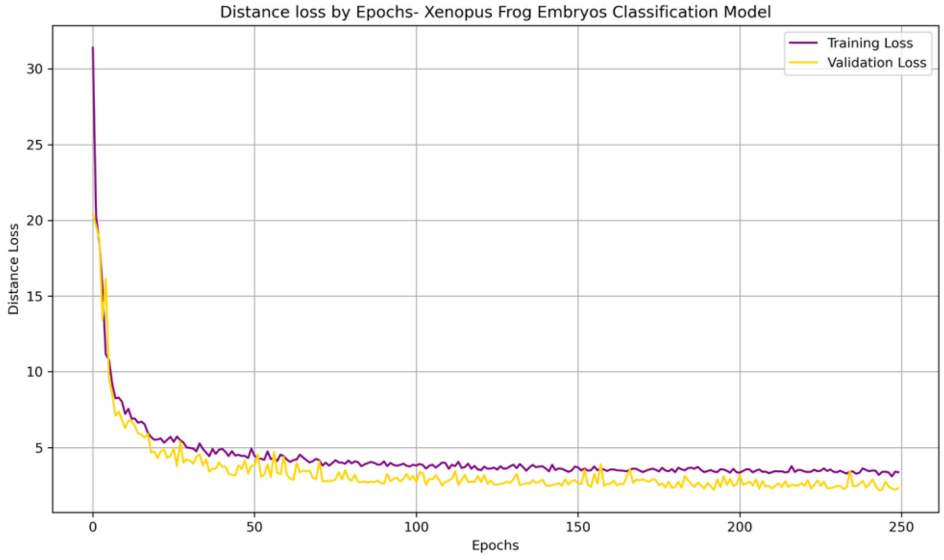

Figure 19. Distance loss graph

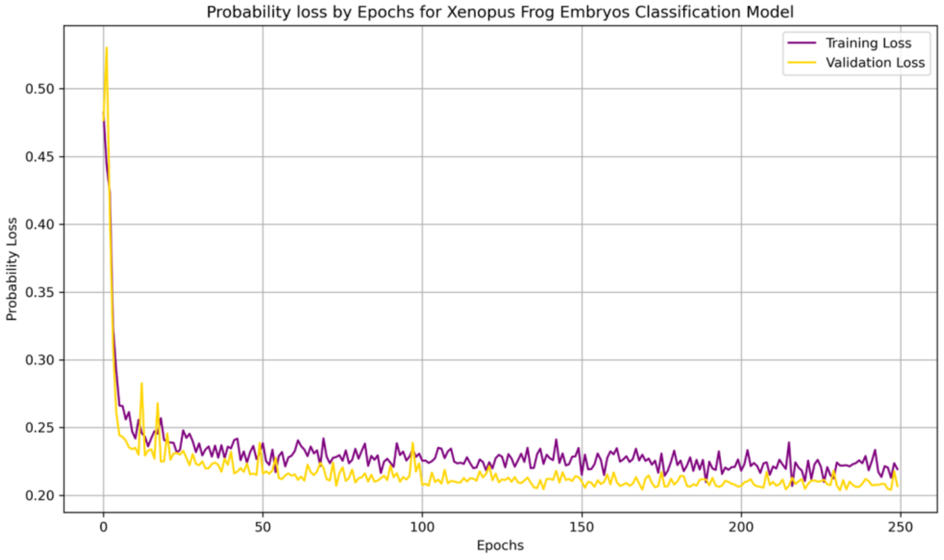

Figure 20. Probability loss graph

- Distance Loss for both training and validation losses started around 30 and decreased to 5 by epochs 50, 100, 150, 200, 250.

- Probability Loss for training and validation started at .5 and dropped sharply to around 0.25 by epoch 10.

The training and validation curves for both losses progressed closely across all epochs, representing the model's consistent learning without major overfitting.

Evaluation Metrics Tests & Results

- Precision = TP / (TP + FP)
- Recall = TP / (TP + FN)
- F1 = (2 × Precision × Recall) / (Precision + Recall)
- Accuracy = TP / (TP + FP + FN)

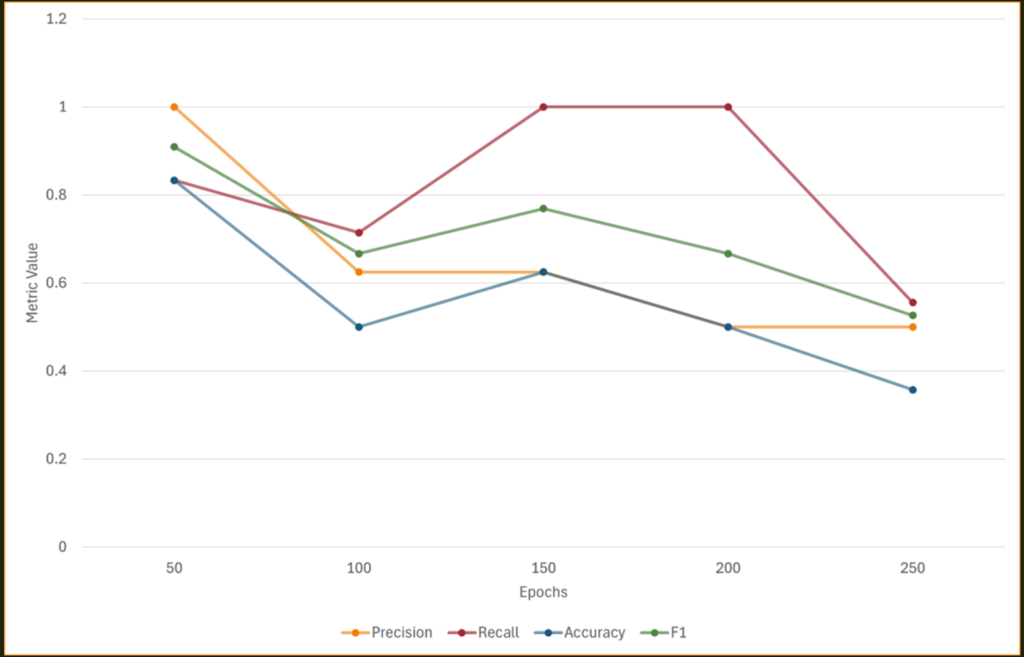

Figure 21. Metrics with IOU 0.4 at Different Epochs

Multiclass Prediction

- Our model had about a 21% accuracy for predicting unfertilized/abnormal and for split-once, two cell embryos

- However, it had 81% and 98% accuracies for predicting split-twice, four cell and split-thrice, eight cell embryos, respectively

- Our test images also included some *Xenopus tropicalis*, whose embryos are similar but smaller

File	Count	Pred	Accuracy
0	212	212	58.96%
1	154	154	52.27%
2	221	221	50.68%
3	166	166	51.20%
4	135	135	67.78%
5	223	223	50.90%
.	.	.	.
.	.	.	.
.	.	.	.
20	188	188	50.27%
21	153	153	49.51%
22	203	203	59.36%
23	334	334	59.51%
24	273	273	64.65%
Total	**5252**	**5223**	**55.54%**

Multiclass Prediction

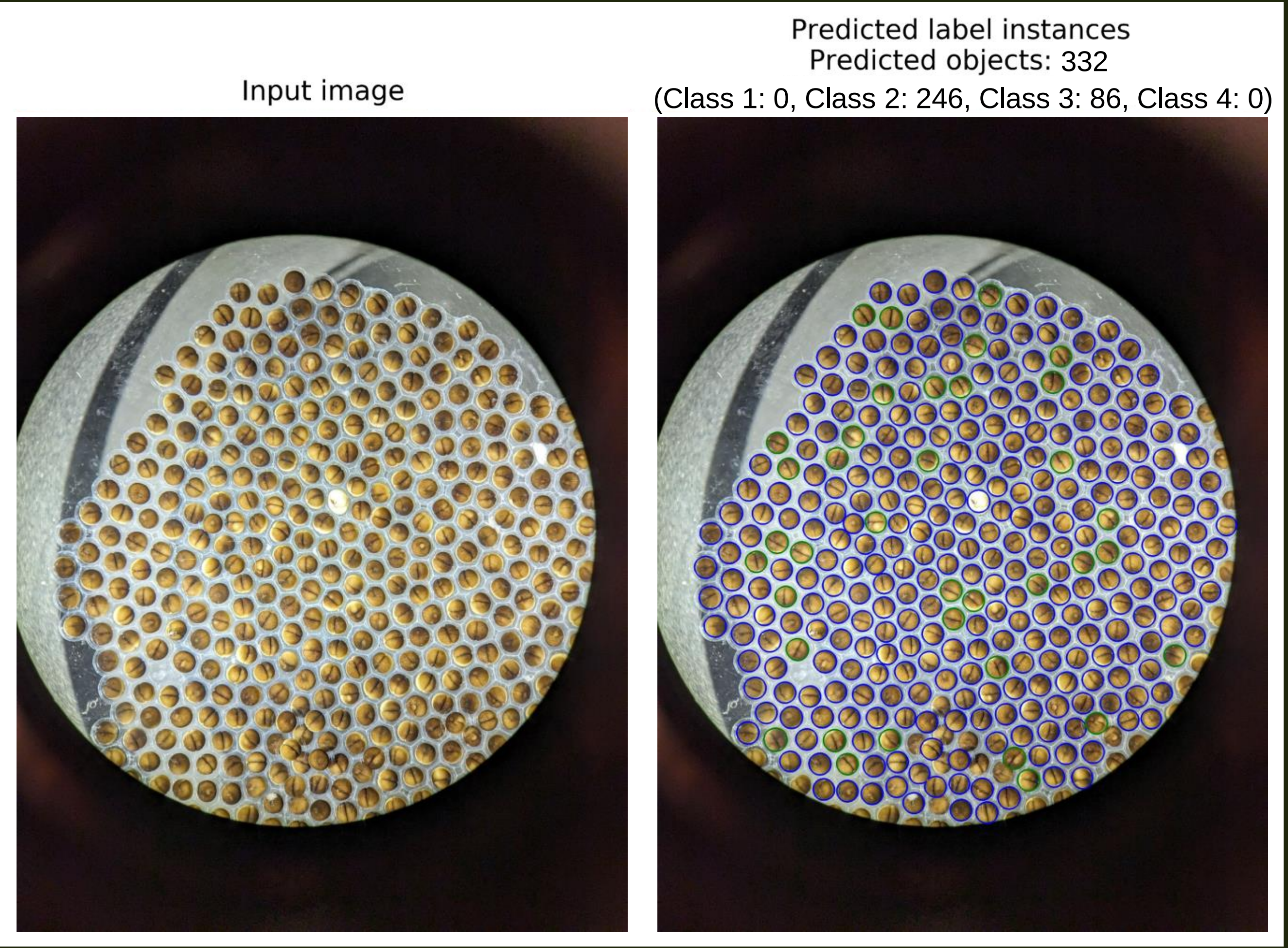

Figure 22.
59% Accuracy

Multiclass Prediction

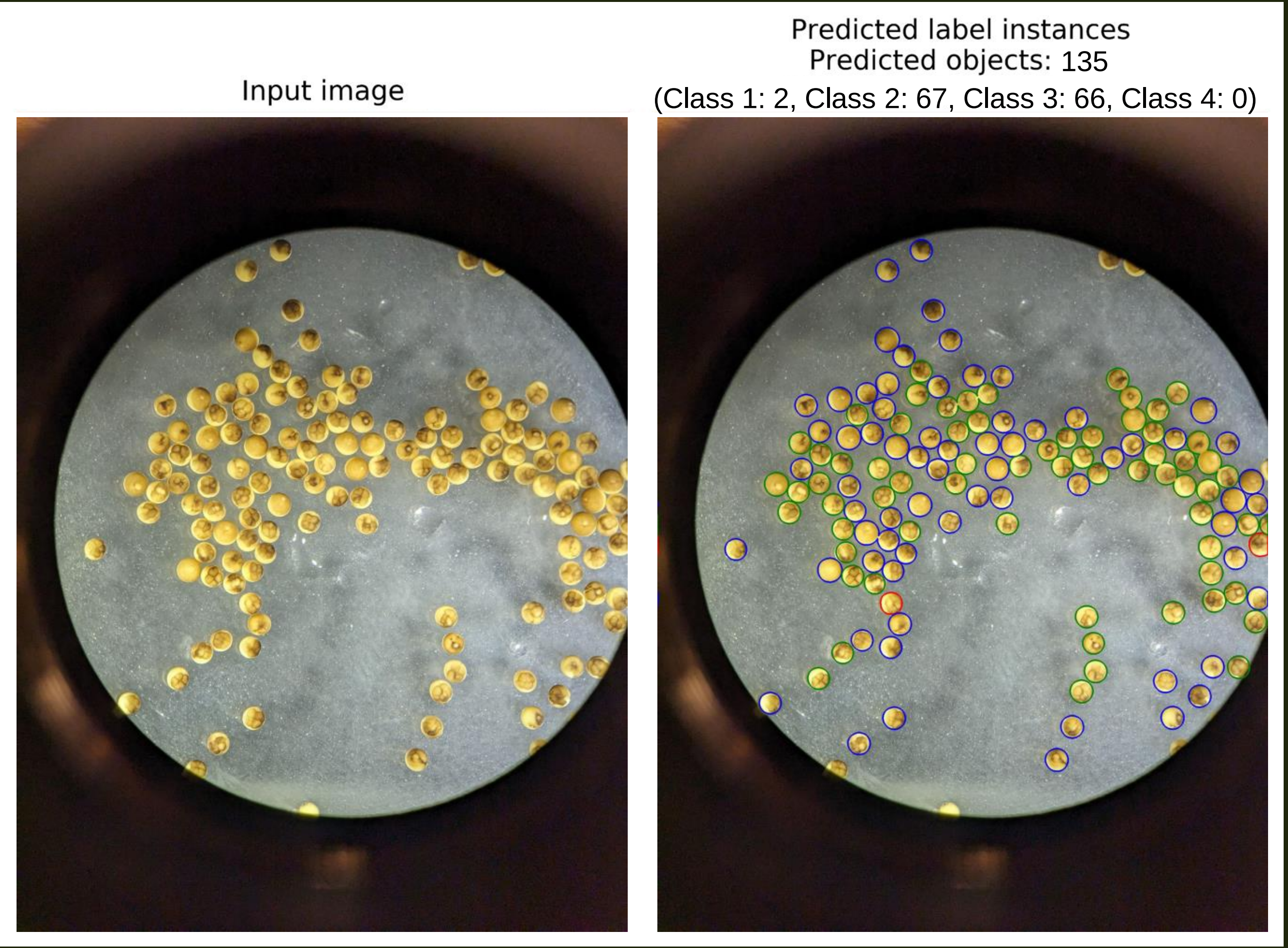

Figure 23.
67% Accuracy

Multiclass Prediction

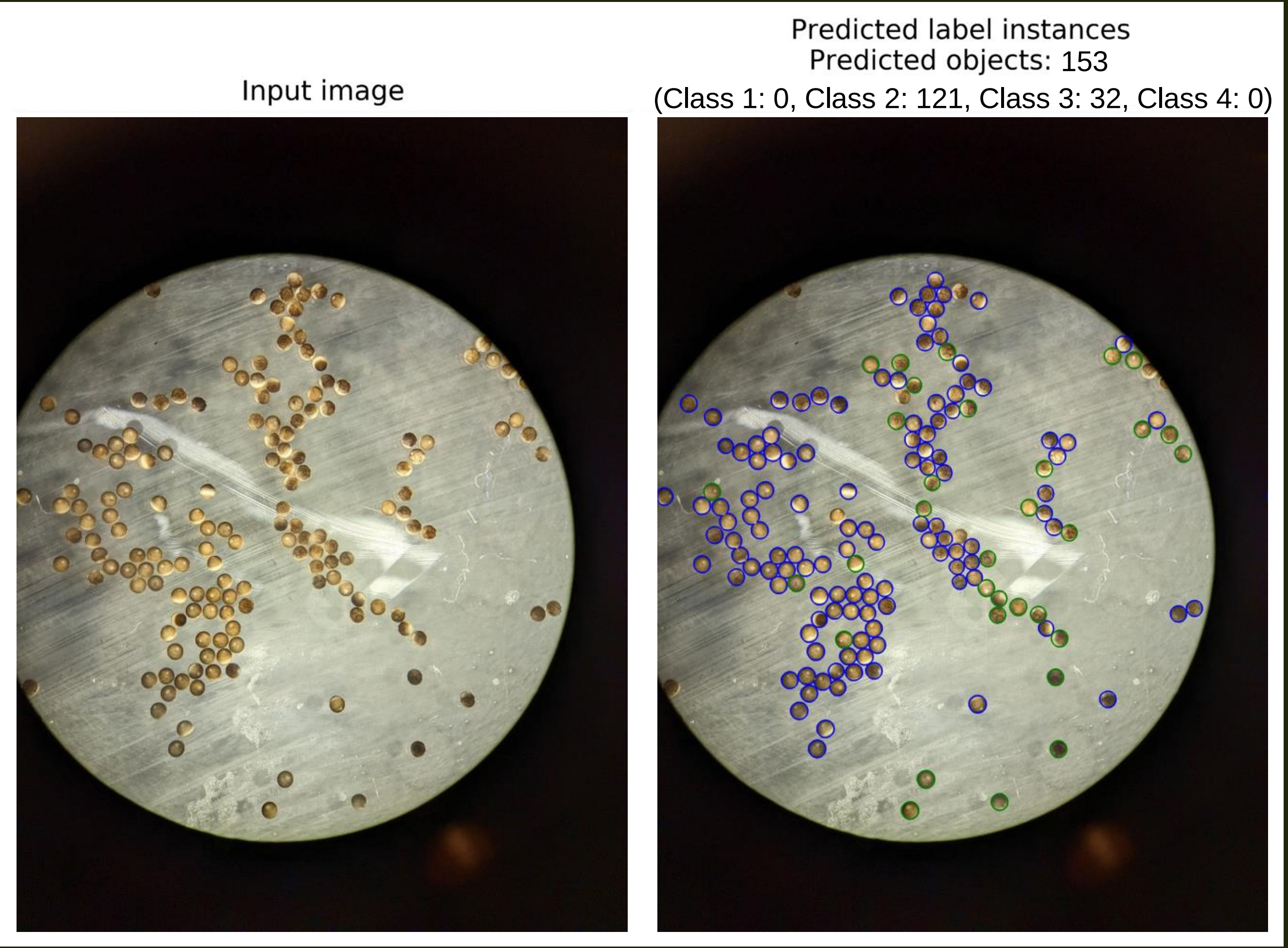

Figure 24.
Xenopus tropicalis
0% Precision and Recall

Graphical User Interface (GUI)

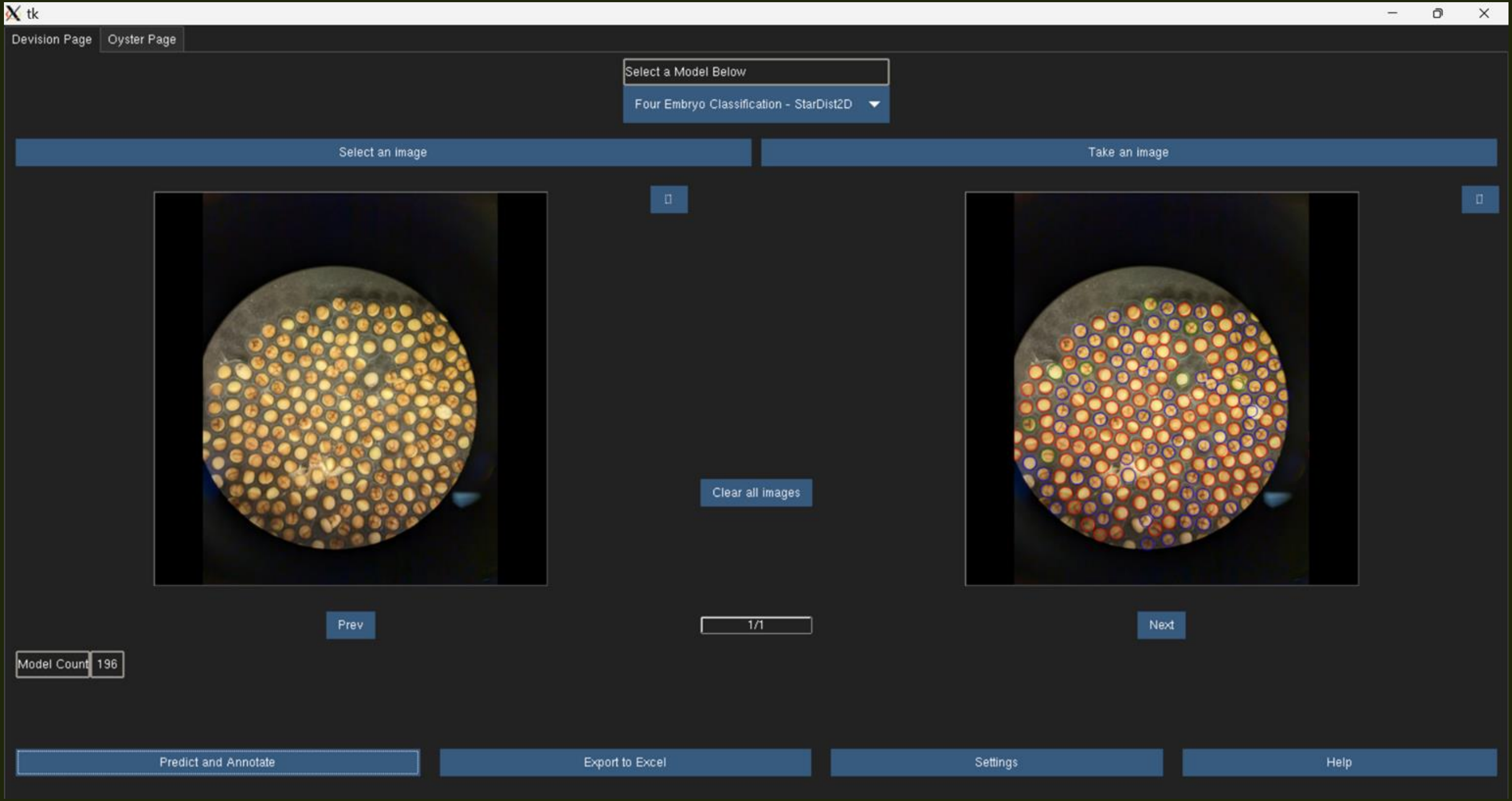

Figure 25. GUI

Future Work

- Image augmentation to increase training set size
- Create a synthetic data set oversampling the minority class
- Achieve accuracy rate comparable to that of previous semesters
- Write an article about StarDist on this specific classification model

Figure 26. Xenopus frog

Works Referenced

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