Electronic Supplementary Material (ESI) for Lab on a Chip.

Deep Learning Detector for High Precision Monitoring of Cell Encapsulation Statistics in Microfluidic Droplets

Supporting document

Karl Gardner, Md Mezbah Uddin, Linh Tran, Thanh Pham, Siva Vanapalli, Wei Li

Department of Chemical Engineering
Texas Tech University, 807 Canton Ave
Lubbock Texas, USA
Corresponding authors: siva.vanapalli@ttu.edu, wei.li@ttu.edu

Experimental Setup

The experimental setup included two microfluidic pumps (PHD 2000, Harvard Apparatus, Massachusetts, USA), a microscope stage system (Nikon eclipse TiU), a high-powered camera (Phantom v710 12-bit, Vision Research) and a desktop computer to capture high quality images from the experiment (Fig. S1). There are two syringe pumps for both the dispersed and continuous phase with cell suspension and fluorinated oil respectively. The fluid suspension flows through a syringe needle connected to a 1.5 mm tubing (inside diameter, tygon), finally passing into the inlets of the microfluidic device. The microscope stage holds the droplet generator while the digital camera is connected through the computer and optical lens. This setup allows for the generated droplets in the expansion chamber to be effortlessly visualized for subsequent analysis and evaluation.

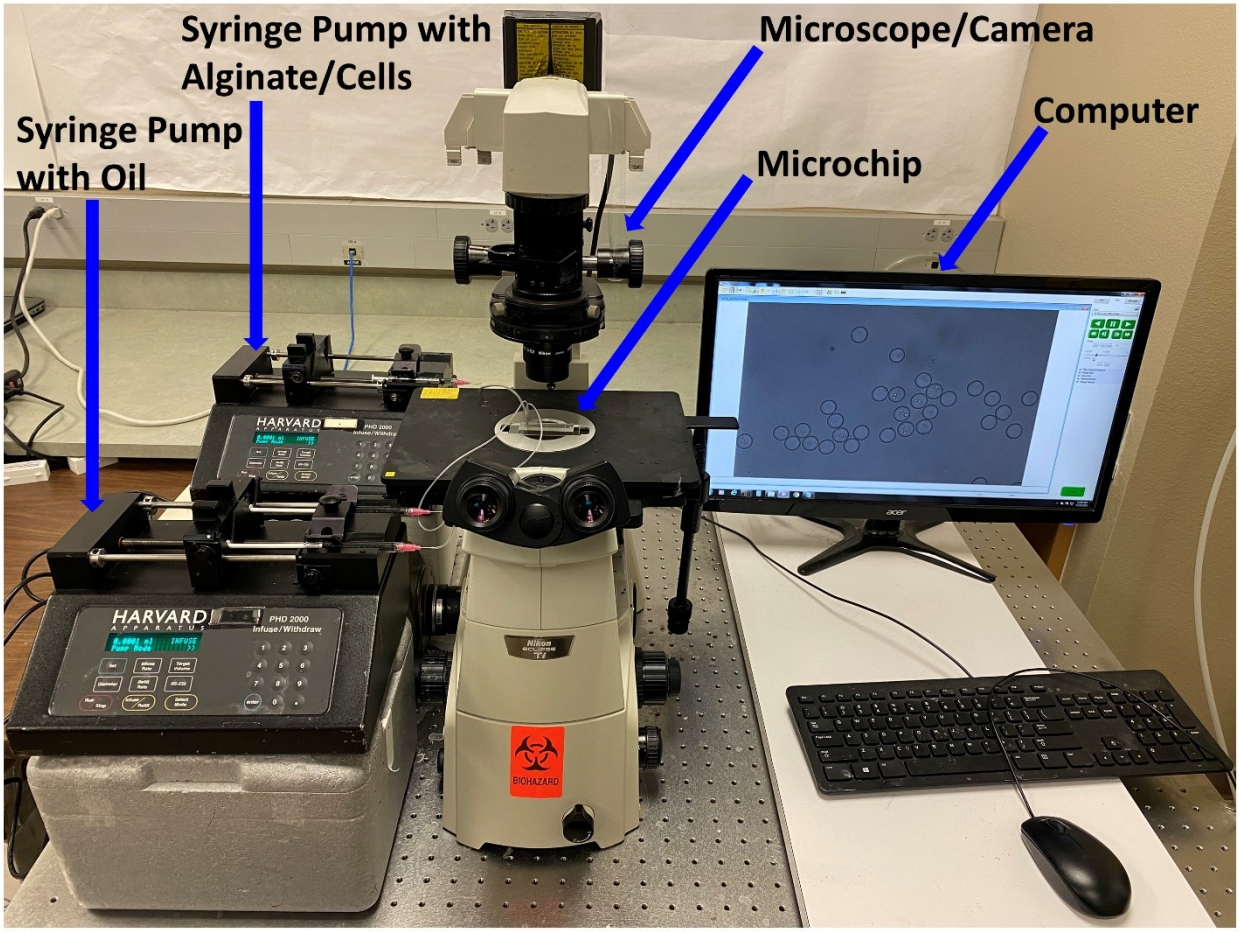


Figure S1. The experimental setup used to capture droplet generation videos in the expansion region. The setup includes a microfluidic device, a high-powered microscope and camera, a syringe pump containing fluorinated oil, a syringe pump containing an alginate cell suspension, and a computer to save the captured images.

Droplet Generation

The microfluidic flow rate for droplet generation was 750 µL/hr and 50 µL/hr for continuous and dispersed phase respectively. A video captured at 400 FPS was used to analyze the droplet generation process in Fig. S2. Here, the images are spaced 2.5 ms apart with the droplet sheared from the continuous phase and gradually moving towards the left. The generated droplet contains three PC3 cells in an alginate solution. The droplet takes around 37.5 ms to reach to the end of the image.

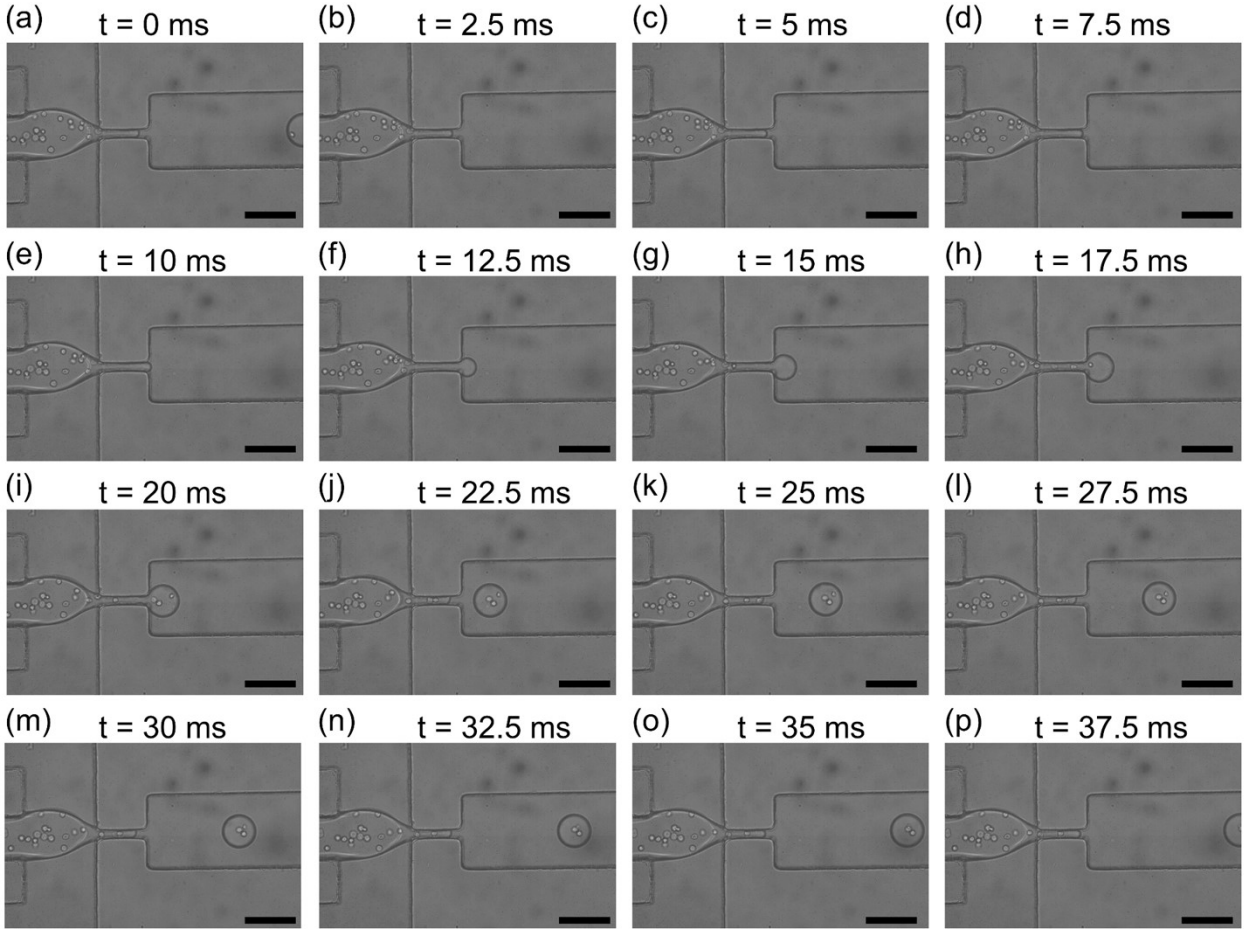


Figure S2. The droplet generation process for one droplet containing three cells. The images are spaced 2.5 ms apart with the droplet moving at a gradual pace. The microscope view of the orifice is at magnification 20x. Scale bar: 150 µm

YOLOv3 Architecture

The original YOLOv3 software was published in darknet, an open-source neural network framework written in the c programming language [1]. The paper provided for this object detection architecture gives a detailed table of only the feature extractor called Darknet-53 but does not outline the entire architecture [2]. Since the published paper in 2018, there has been other implementations in the python programming language, including the popular YOLOv3 by ultralytics. The full architecture is provided in Fig. S3 with both Darknet-53 feature extractor and output layers [3].

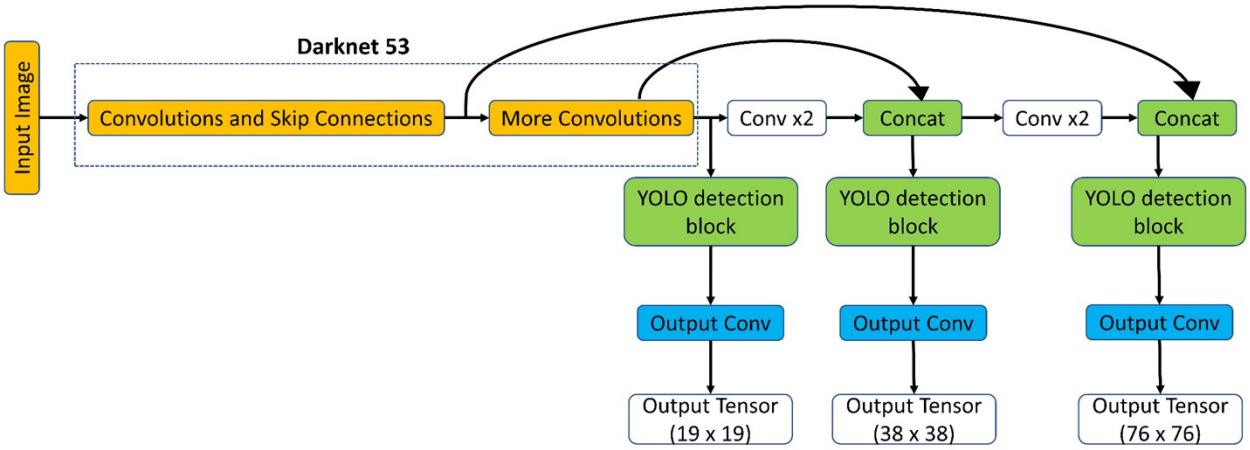


Figure S3. The network architecture for yolov3 used for both the droplet and cell model to detect both droplets containing cells and the individual cells in the droplets.

YOLOv5 Architecture

The YOLOv5 architecture by ultralytics is written exclusively in python and its external libraries, e.g., PyTorch[4, 5]. The network pipeline in Fig. S4, from an issue posted on GitHub, consists of a cross stage partial network (CSPNet) as its backbone, a path aggregation network (PANet) as its neck, and an output layer generating three different sizes (18 x 18, 36 x 36, and 72 x 72) [6]. The CSPNet solves the problems of repeated gradient information in large-scale backbones [7], PANet improves the propagation of low-level features [8], and the output layer allows the model to handle small, medium, and large objects [2].

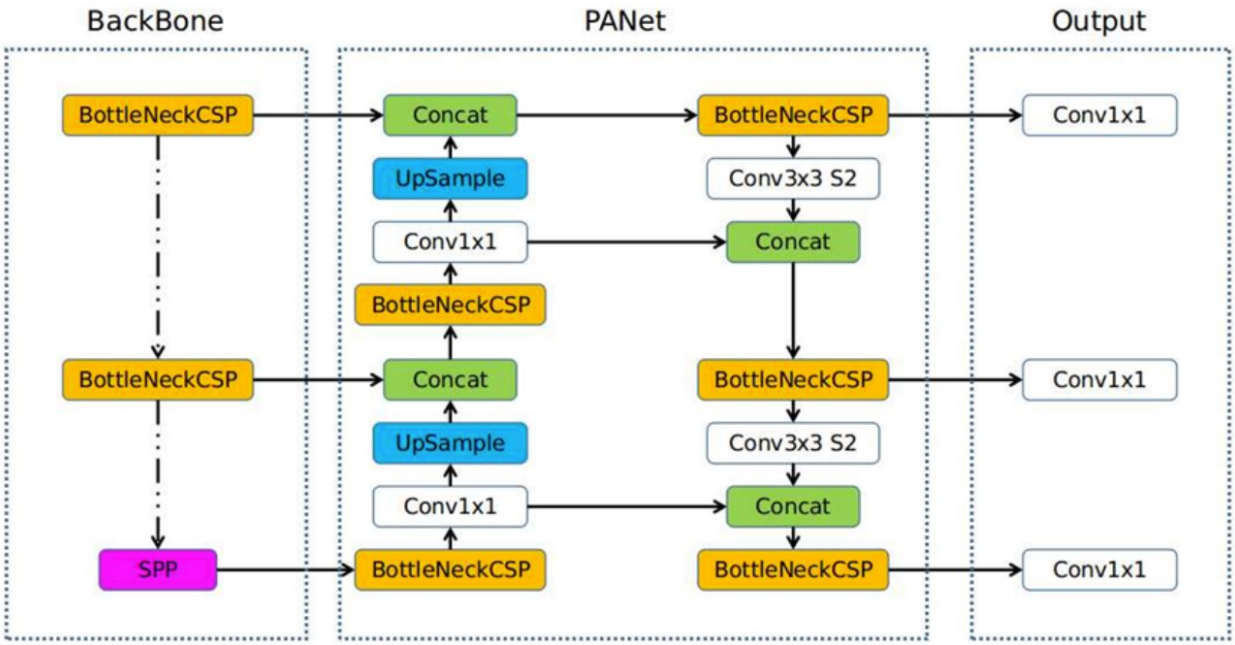


Figure S4. The network architecture for YOLOv5 used for the droplet model. The three sections of the architecture consist of the model backbone, PANet, and Output.

Cell Model ML Metrics

Performance metrics for the cell model is shown in Fig. S5 with validation (a, b) and test (c, d) set metrics. The precision recall curve illustrates that the precision decreases as recall increases.

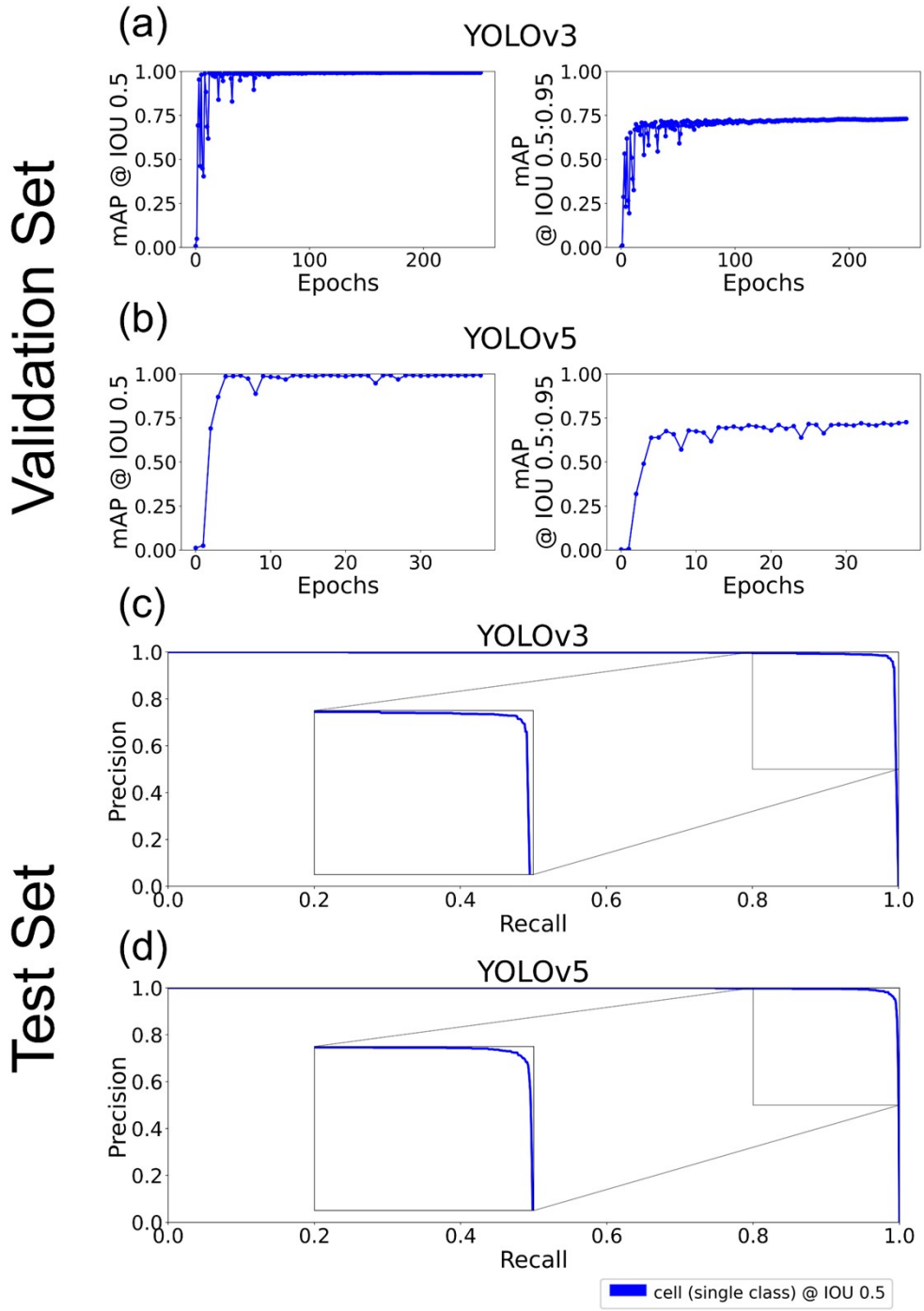
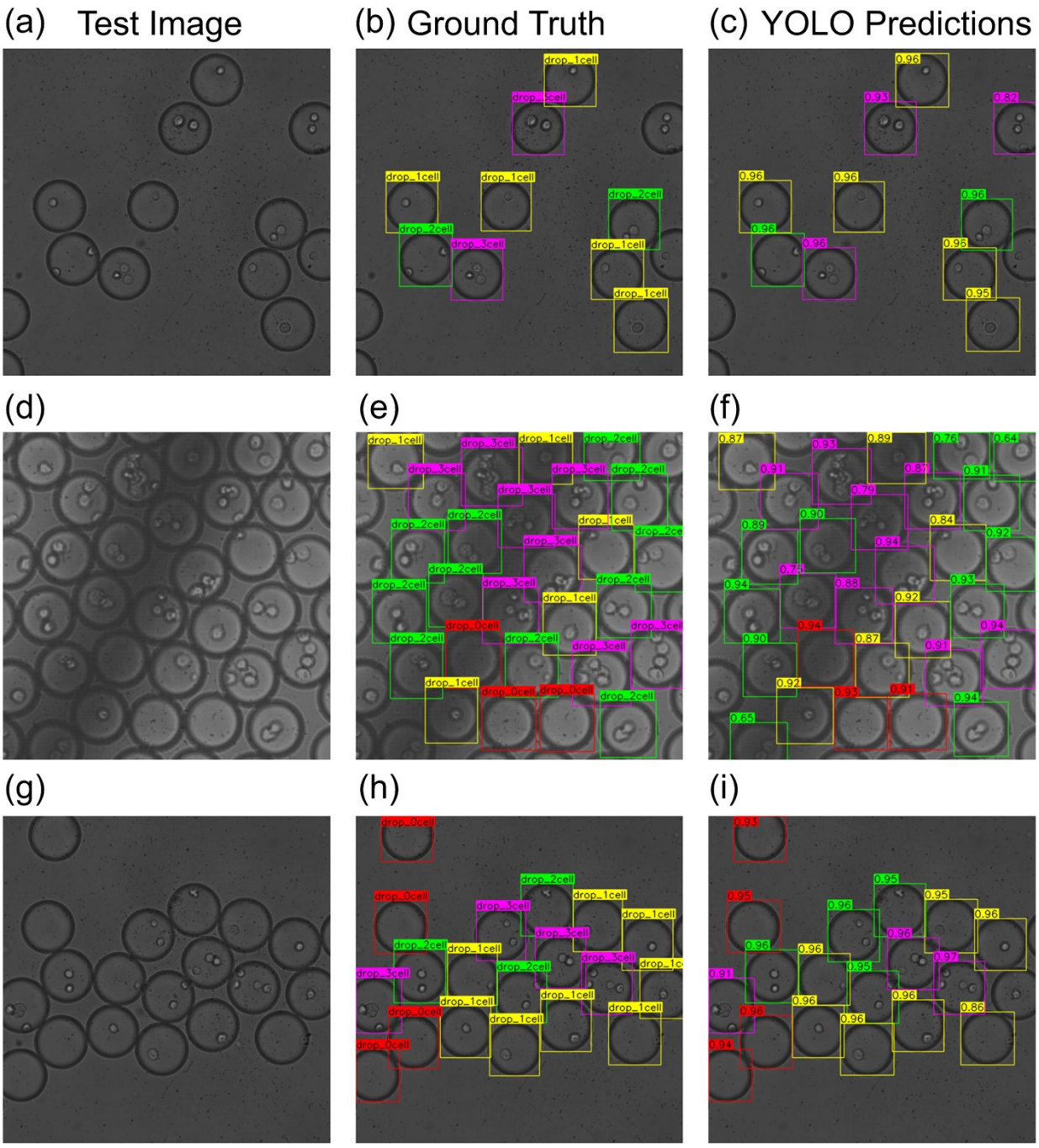


Figure S5. Cell model validation (a, b) and test (c, d) set metrics for both YOLOv3 and YOLOv5 networks. The validation metrics show the mAP at 0.5 IOU threshold and 0.5-0.95 IOU threshold for all epochs trained with both models. The test set metrics display the precision recall curve at 0.5 IOU threshold for the cell class in both models.

Droplet Model Predictions

The predictions can be visualized with the confidence value and each label represented as a different color. Six random (pseudo-random pool of numbers) examples from the test set are illustrated in Fig. S6 with the input image, ground truth labels, and predictions arranged as left, middle, right. The confidence value on the top of each colored box represents the objectness score of the prediction.



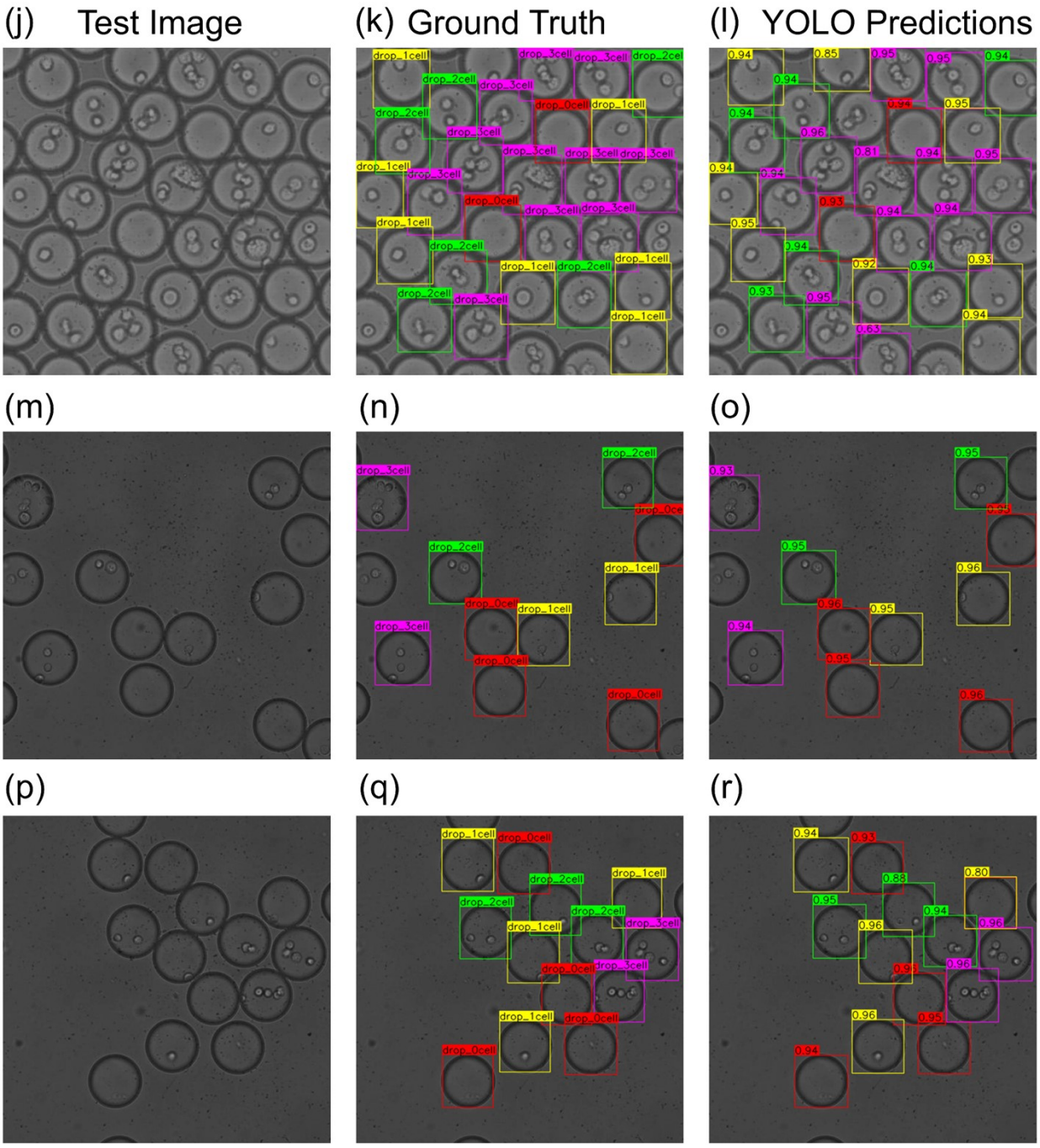


Figure S6. Droplet model predictions with input image on left, ground truth labels in middle, and predictions on right for six random test set examples (a-r). The predictions were ran using the YOLOv5 model weights while the NMS was conducted with an IOU threshold of 0.45. The confidence threshold for plotting bounding boxes was set to 0.6.

Cell Model Predictions

Nine random (pseudo-random pool of numbers) examples from the test set are illustrated in Fig. S7 with ground truth labels (green), predictions (red), and confidence values (cyan) provided.

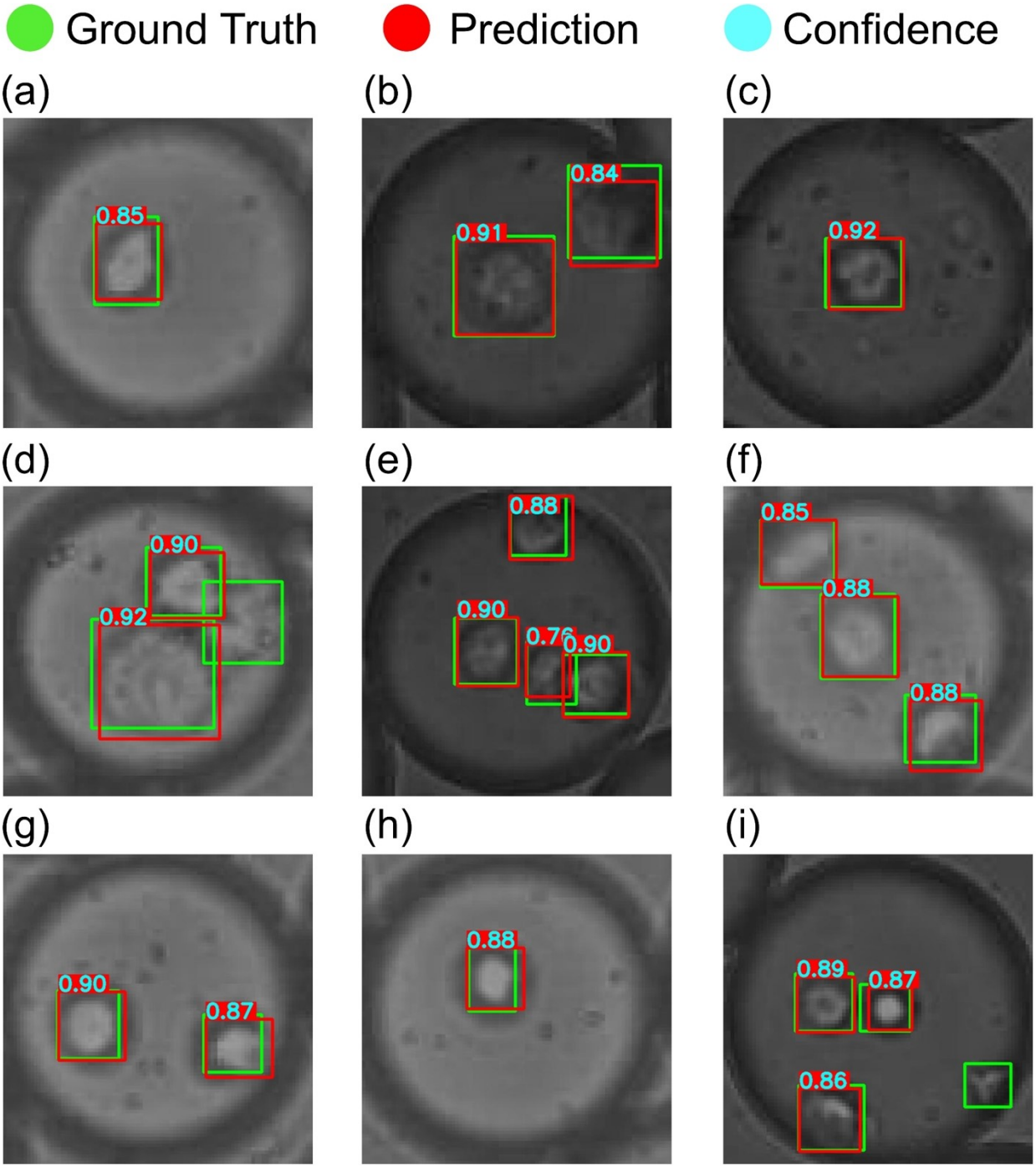


Figure S7. Cell model images with ground truth labels (green), YOLOv5 predictions (red), and confidence values (cyan) from nine random test set examples (a-i). NMS was conducted with an IOU threshold of 0.6, and the confidence threshold for plotting detections was fixed to 0.25.

Hand Counting Comparisons

To verify the statistics match with a smaller number of images, a random sequential batch of 50 images (equivalent to 0.5 seconds) from two separate trials in the production set is analyzed. The droplet proportions from the 100 images are manually counted and compared to the droplet proportions exported from the YOLOv5 model. In Fig. S8 the comparison for both trials in (a) and (b) illustrate that the YOLO droplet totals for a smaller set of images agree with hand counted proportions.

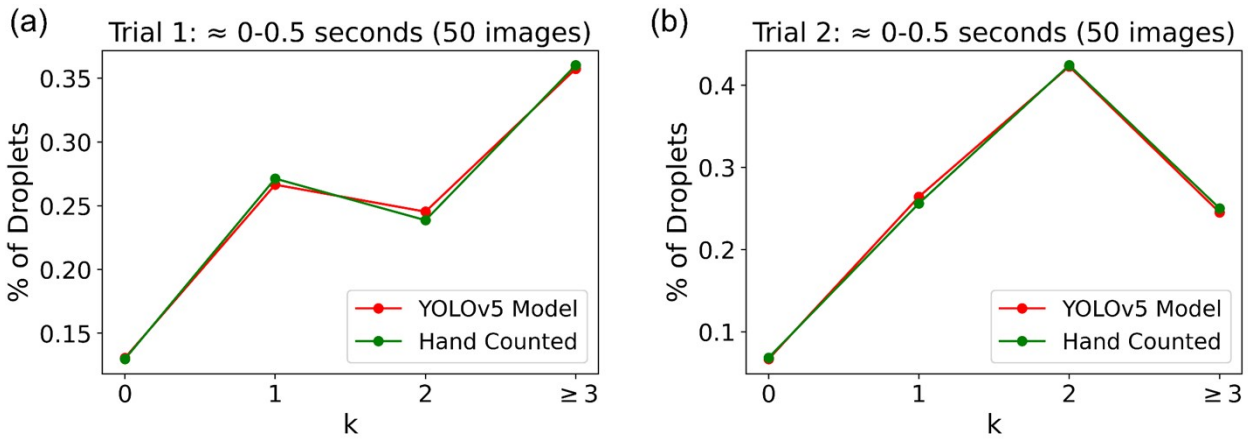


Figure S8. The fraction of droplets containing zero, one, two, or greater than two cells. Droplets are counted from YOLOv5 predictions (red) and by hand (green) for a total of 100 images over two trials. The images were preprocessed from the original video (taken at 100 FPS) resulting in a 0.5 second time frame for each trial. The NMS for the YOLOv5 model was completed with an IOU threshold of 0.45 while the confidence threshold was set to 0.6.

Performance Metrics

In an object detection model, there are four performance metrics that are evaluated on a test set to measure how well the predictions compare to ground truth labels. These four metrics are precision, recall, mAP at an IOU threshold of 0.5, and mAP at an IOU threshold of 0.5 through 0.95. Specifically, the IOU threshold provides a value to set the boundary of an incorrect or correct prediction. The mAP metric can use multiple IOU thresholds, then average the results of all the thresholds used. For both YOLOv3 and YOLOv5 these four metrics are computed for each class in Table S1 and S2. The rows represent each class with the average of all classes in the last row while the columns define the specific type of metric. The mAP @ 0.5:0.95 IOU for all classes is equivalent to two decimal places for both YOLOv3 and YOLOv5 models, in addition to having similar values for the other three metrics.

Table S1. Droplet Model Test Set Performance with YOLOv3 and YOLOv5

Class/Metric	Precision	Recall	mAP (@ 0.5 IOU)	mAP (@ 0.5:0.95 IOU)
		YOLOv3		
Drop_0cell	0.97	0.96	0.98	0.92
Drop_1cell	0.93	0.95	0.96	0.9
Drop_2cell	0.91	0.92	0.95	0.9
Drop_3cell	0.96	0.93	0.96	0.91
All	0.94	0.94	0.97	0.91
		YOLOv5		
Drop_0cell	0.97	0.96	0.99	0.93
Drop_1cell	0.94	0.96	0.97	0.91
Drop_2cell	0.9	0.93	0.95	0.9
Drop_3cell	0.94	0.94	0.97	0.91
All	0.94	0.95	0.97	0.91

Table S2. Cell Model Test Set Performance with YOLOv3 and YOLOv5

Class/Metric	Precision	Recall	mAP (@ 0.5 IOU)	mAP (@ 0.5:0.95 IOU)
		YOLOv3		
Cell	0.98	0.99	0.99	0.71
		YOLOv5		
Cell	0.98	0.99	0.99	0.71

References

1. Redmon, J., *Darknet: Open Source Neural Networks in C*. 2013-2016, https://github.com/pjreddie/darknet: http://pjreddie.com/darknet/.
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4. Jocher, G., et al., *ultralytics/yolov5: v6.0 YOLOv5n 'Nano' models, Roboflow integration, TensorFlow export, OpenCV DNN support*. 2021, Zenodo: https://github.com/ultralytics/yolov5.
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6. Jocher, G. and seekFire. *Yolov5m architecture issue #6094*. 2021 [cited 2022; Available from: https://github.com/ultralytics/yolov5/issues/6094.
7. Wang, C.-Y., et al., *CSPNet: A New Backbone that can Enhance Learning Capability of CNN*. 2020. p. 1571-1580.
8. Wang, K., et al., *PANet: Few-Shot Image Semantic Segmentation with Prototype Alignment.* CoRR, 2019. **abs/1908.06391**.