Advancing Tissue Culture Analysis: A Novel Machine Learning Algorithm for Cellular Viability

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Bridge UnderGrad Science (BUGS) Summer Research Program

SOFTWARE TOOLS • 3D TISSUE CULTURE • SEGMENTATION

Abstract

SAAVY, a **S**egmentation **A**lgorithm to **A**ssess the **V**iabilit**Y** of 3D cell cultures, is a tool we created to allow researchers to analyze 3D spheroid cultures with high throughput and accuracy, relying solely on brightfield microscopy images. Many current tools such as CellTiter-Glo viability assay rely on lysing the spheroids reducing the available options for analysis to only endpoint. Such limitations can be avoided through automating the viability analysis using imaging combined with computer vision. SAAVY combines a neural net-based segmentation algorithm and custom analysis tools to provide automated nondestructive viability analysis. We tested SAAVY against human experts and show comparative performance with estimating spheroid viability. Furthermore, we demonstrate a practical use of SAAVY by longitudinally tracking spheroid viability over time.
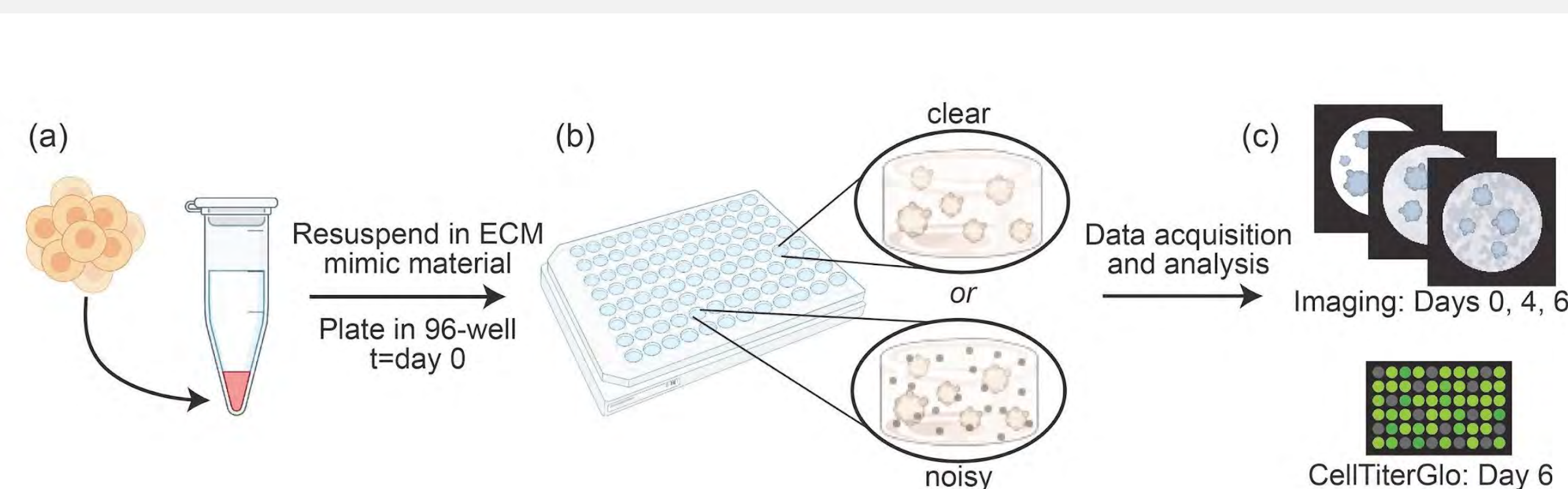
Highlights

- Consistent performance even in a high noise environment
- Quick analysis ~ 120s for 420 images
- Outputs more than just viability, with other metrics such as average area, eccentricity, and average intensity
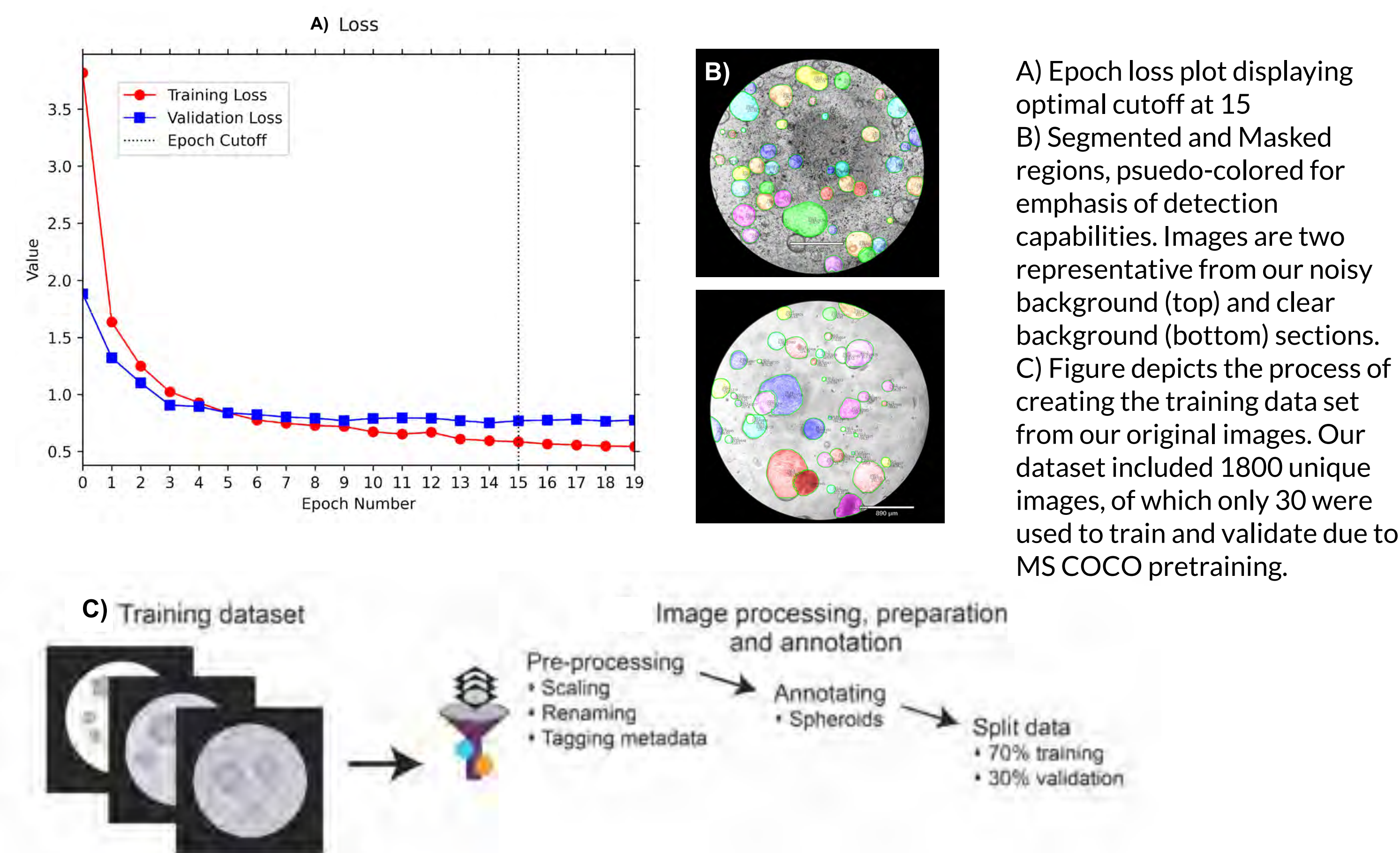
Methods

We first train SAAVY using a pre-existing image set from MS COCO pretrained general model for transfer learning. To fine tune Mask-RCNN, we replace the region proposal network and mask network with new F-RCNN networks and train for 20 epochs saving model checkpoints. We trained for 20 epochs. Based on analysis, we used the 15th checkpoint for image production due to continuous loss after this point. Using the training dataset detailed above, we annotate the 24 training images using VIA Image Annotator (2.0.11, Oxford) to specifically identify cell spheroids. We digitally resize validation images to 1024x1024 before viability analysis. Total computational time for SAAVY on the 416-image set is 2 minutes and 5 seconds (running an RTX 3080 and Intel Core i9-10850K stock).
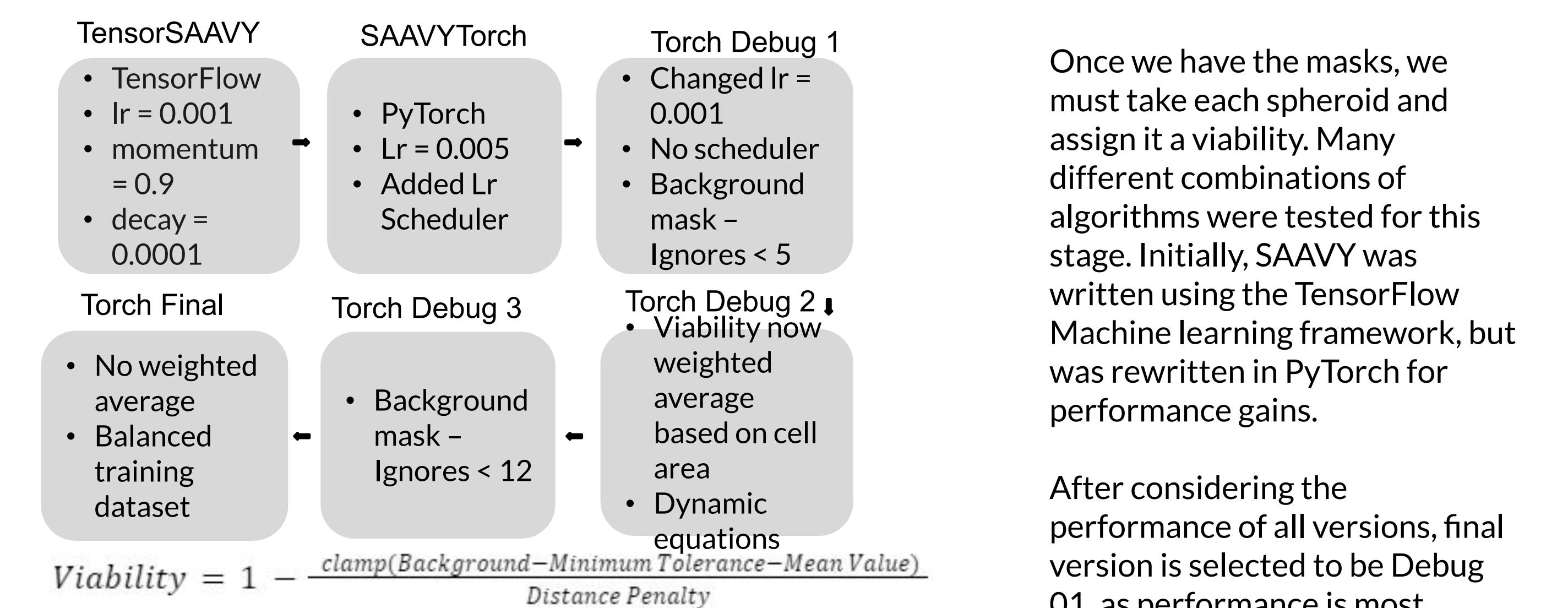
Graphical Abstract

(a) Resuspend in ECM mimic material / Plate in 96-well t=day 0
(b) clear or noisy
(c) Data acquisition and analysis — Imaging: Days 0, 4, 6 / CellTiterGlo: Day 6

SAAVY Development: Machine Learning Segmentation

Creation of fine-tuned Mask-RCNN model pretrained from MS COCO

A) Loss

A) Epoch loss plot displaying optimal cutoff at 15
B) Segmented and Masked regions, psuedo-colored for emphasis of detection capabilities. Images are two representative from our noisy background (top) and clear background (bottom) sections.
C) Figure depicts the process of creating the training data set from our original images. Our dataset included 1800 unique images, of which only 30 were used to train and validate due to MS COCO pretraining.

C) Training dataset
Pre-processing • Scaling • Renaming • Tagging metadata → Image processing, preparation and annotation — Annotating • Spheroids → Split data • 70% training • 30% validation

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SAAVY Optimization: Viability Algorithm

Developing the analysis component that ingests segmented masks

TensorSAAVY
- TensorFlow
- lr = 0.001
- momentum = 0.9
- decay = 0.0001

SAAVYTorch
- PyTorch
- Lr = 0.005
- Added Lr Scheduler

Torch Debug 1
- Changed lr = 0.001
- No scheduler
- Background mask – Ignores < 5

Torch Final
- No weighted average
- Balanced training dataset

Torch Debug 3
- Background mask – Ignores < 12

Torch Debug 2
- Viability now weighted average based on cell area
- Dynamic equations

Once we have the masks, we must take each spheroid and assign it a viability. Many different combinations of algorithms were tested for this stage. Initially, SAAVY was written using the TensorFlow Machine learning framework, but was rewritten in PyTorch for performance gains.

After considering the performance of all versions, final version is selected to be Debug 01, as performance is most comparable to expert.

$$Viability = 1 - \frac{clamp(Background - Minimum\ Tolerance - Mean\ Value)}{Distance\ Penalty}$$

evaluation	metric	SAAVY	SAAVY v1.5	SAAVYTorch	SAAVY v1	SAAVY v2	SAAVY v3	SAAVY v4
ID clear d0	accuracy	0.4	0.4857	0.4857	0.5714	0.485	0.471	0.471
ID clear d4	f1- score	1	1	1	1	1	1	1
ID clear d6	f1- score	1	1	1	1	1	1	1
ID noisy d0	accuracy	0.3	0.314	0.528	0.5857	0.314	0.357	0.357
ID noisy d4	f1- score	0.992	0.992	0.992	0.992	0.992	0.992	0.992
ID noisy d6	f1- score	0.984	0.984	0.984	0.984	0.984	0.984	0.984
LD clear d4	f1- score	1	1	1	1	1	1	1
LD clear d6	f1- score	0.879	0.894	0.8429	0.894	0.8869	0.9107	0.9107
LD noisy d4	f1- score	0.992	0.992	0.992	0.992	0.992	0.992	0.992
LD noisy d6	f1- score	0.938	0.972	0.9137	0.972	0.963	0.9629	0.9629

SAAVY Application: High Throughput Longitudinal Drug-Response Assay

Longevity Analysis of a Therapeutic on a Disease
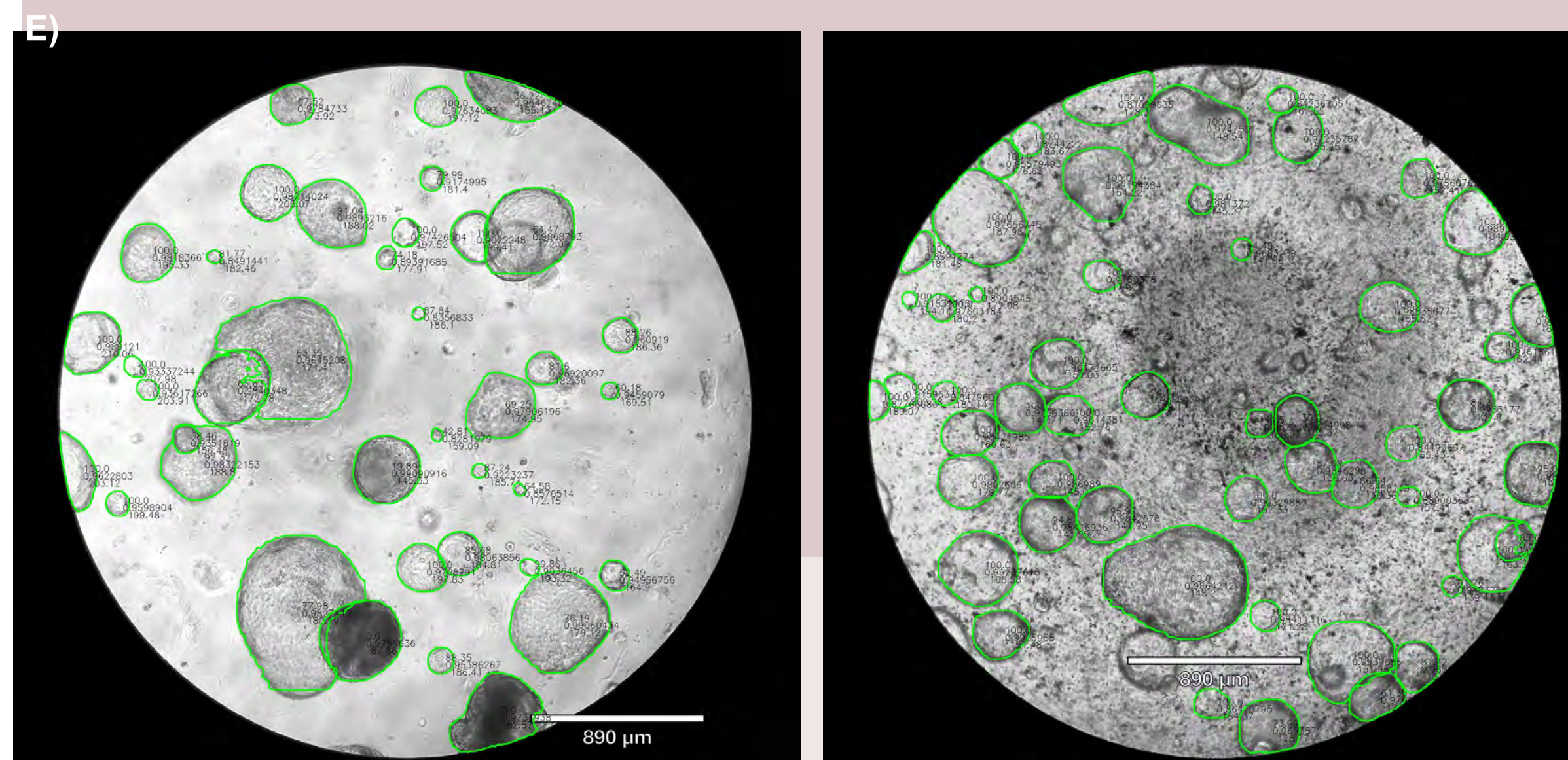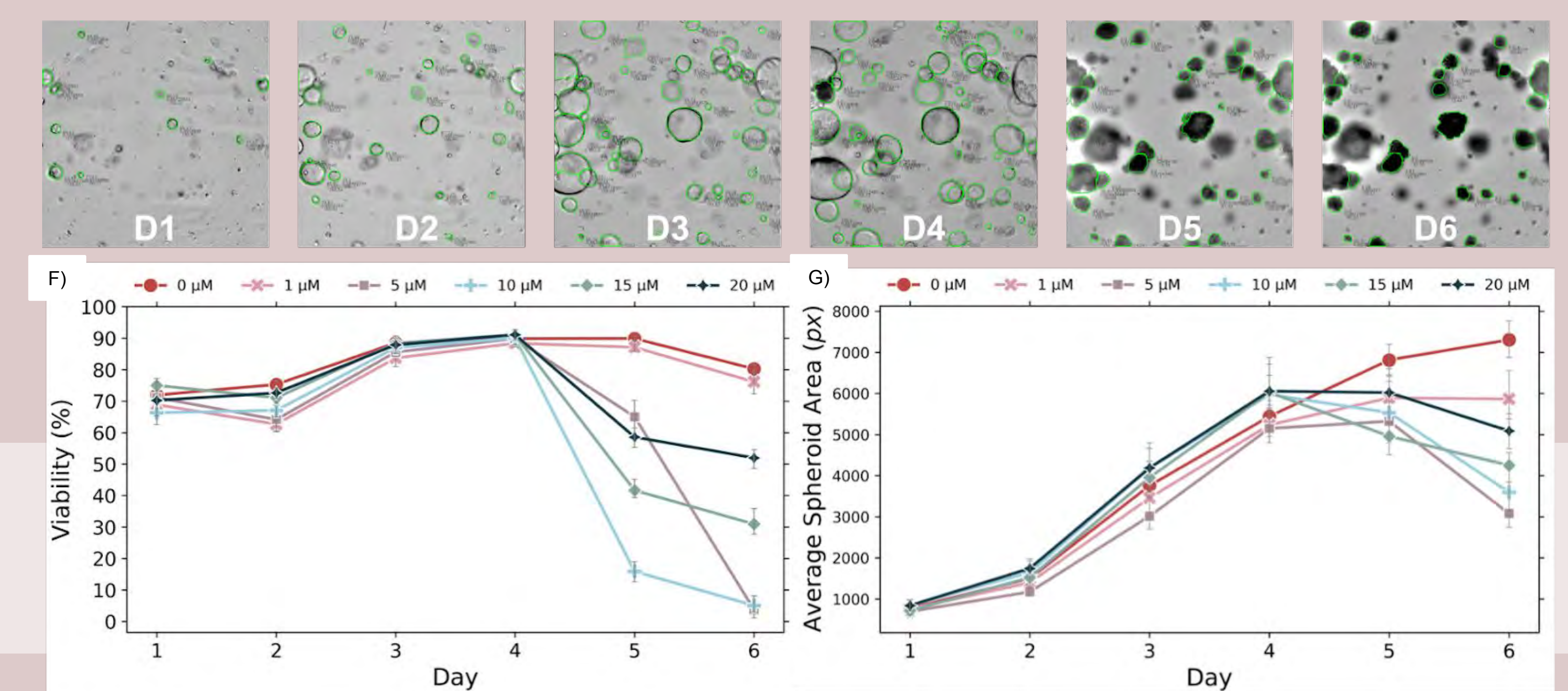

890 µm

Figure E, demonstrates SAAVY Performance even in the presence of high background noise signal (right). Average viability, circularity, and size are exported to CSV for review. **This information is not available from either expert or CTG analysis** and provides a new dimension for analytics.

Viability resolution is improved 50x from Expert to SAAVY Analysis. We explored several potential use cases of interest using pancreatic ductal adenocarcinoma organoids as a representative biological system (shown in figure E). Due to the nature of fine tuning a pretrained model, SAAVY is very easy to adapt to other types of organoids.

Longevity Analysis of a Therapeutic on a Disease

D1 D2 D3 D4 D5 D6

F) 0 µM, 1 µM, 5 µM, 10 µM, 15 µM, 20 µM

G) 0 µM, 1 µM, 5 µM, 10 µM, 15 µM, 20 µM

F) Viability plot of spheroid samples tracked over 6 days during a drug perturbation assay. We see decrease in spheroid viability on days 5 and 6 as noted in the images on the same days. **We prove the successful ability of SAAVY to track viabilities for drug response assays.**

G) Plot of average size of spheroids tracked across the same assay. Plots display complementary information This plot displays decrease in size for visually dead cells. **Thus, SAAVY can provide rate information of spheroid growth during this assay**

Acknowledgements