A deep learning pipeline for segmentation of *Proteus mirabilis* colony patterns

Anjali Doshi*[1], Marian Shaw*[1], Ruxandra Tonea[1], Rosalía Minyety[1], Soonhee Moon[1], Andrew Laine[1], Jia Guo^◇[2,3], Tal Danino^◇[1,4,5]

[1]Department of Biomedical Engineering, [2]Department of Psychiatry, [3]Mortimer B. Zuckerman Mind Brain Behavior Institute, [4]Herbert Irving Comprehensive Cancer Center, [5]Data Science Institute; All affiliated to Columbia University, New York, NY USA
*,^: equal contribution; ◇: corresponding author
Correspondence: tal.danino@columbia.edu, jg3400@columbia.edu

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Background

- *Proteus mirabilis*, a bacterium commonly found in water and soil, can cause infections of the lungs, wounds, and urinary tract. In the USA, it accounts for ~44% of catheter-associated UTIs.[1]
- A critical part of its virulence is Its "swarming motility," a highly coordinated movement propelled by flagella.[2]
- *P. mirabilis* swarming is typically studied through colony development assays in which the bacterium forms a characteristic macroscopic bullseye pattern.[3,4]
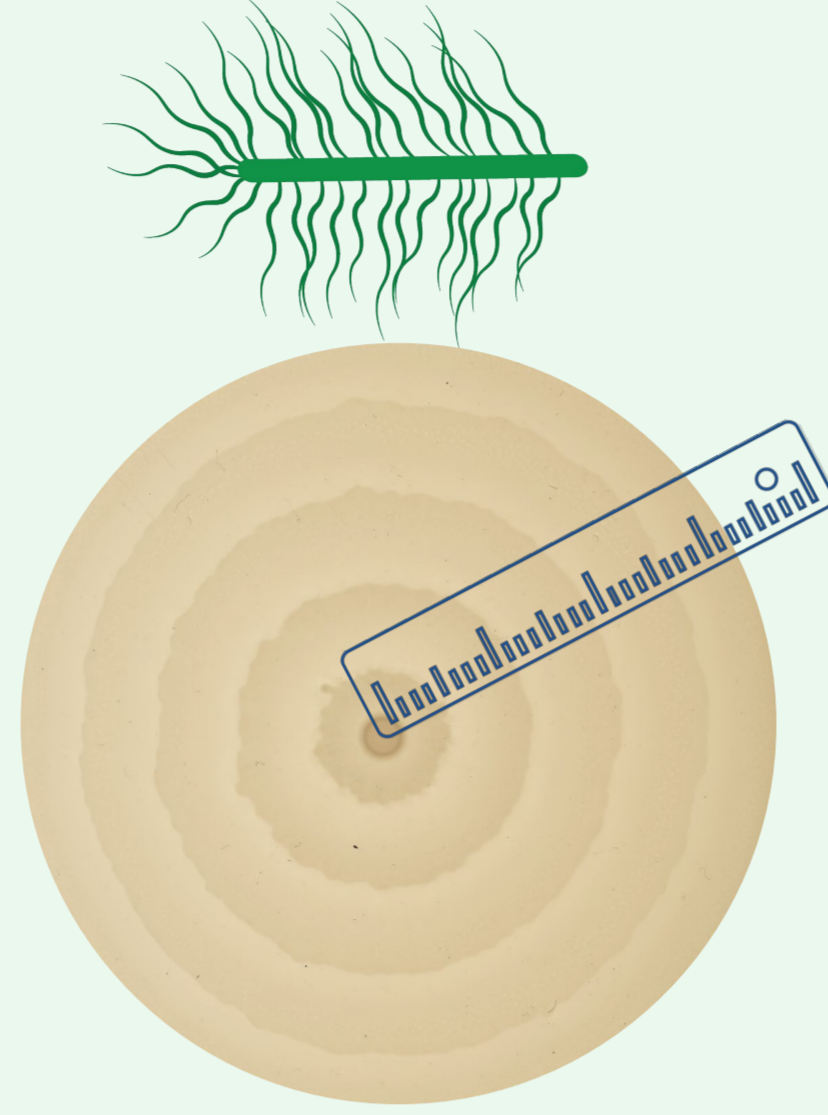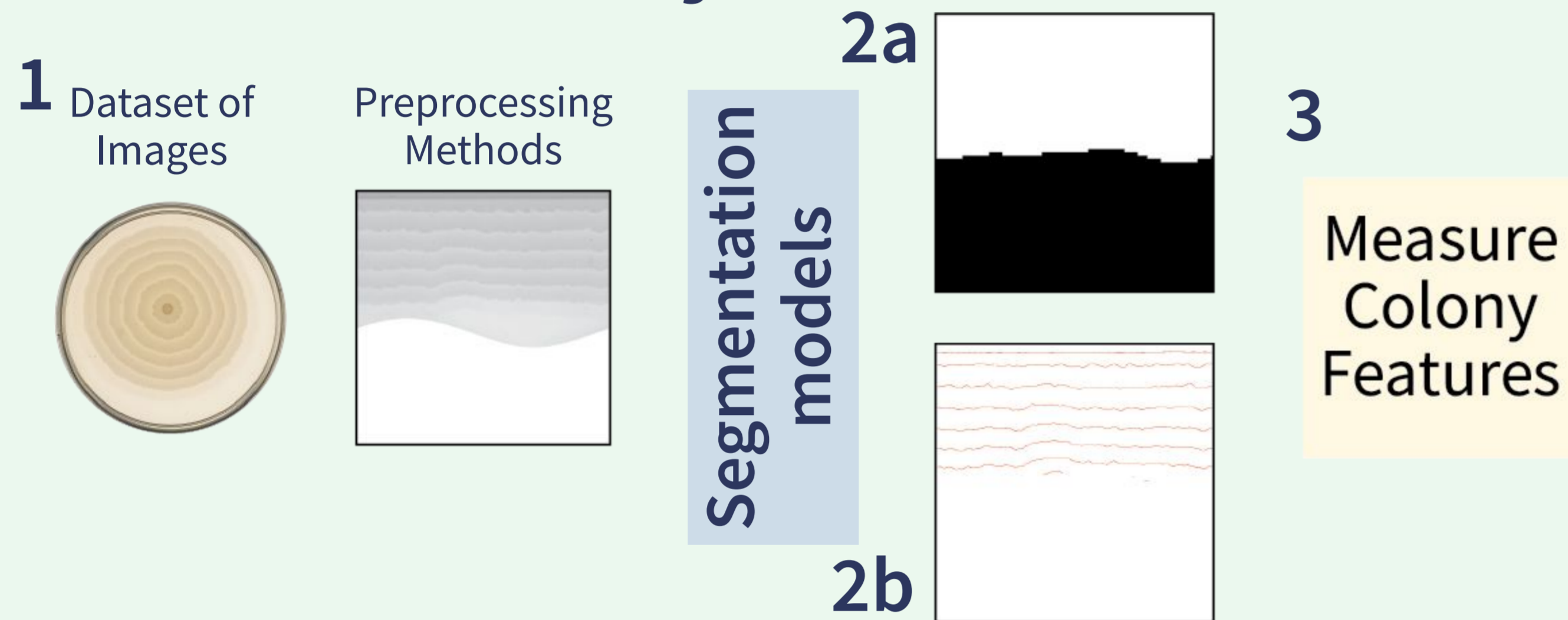
Problem: There is no computational state-of-the-art method for efficient, comprehensive, and scalable analysis of macroscopic *P. mirabilis* colonies.

Highlights

- We built a dual-task pipeline for segmenting *P. mirabilis* colonies, including:
  - Creating the first (to our knowledge) large-scale dataset of *P. mirabilis* colony images
  - Image-processing algorithms for semi-automated preprocessing and ground-truth generation
  - Patch-based segmentation of the colony from background, including faint outer swarm rings, achieving 93.28% test Dice score
  - U-Net segmentation of internal ring boundaries, achieving a 83.24% test Dice score, and post-processing for noise reduction
- We conducted a standard assay investigating swarming under different conditions and showed how the pipeline can be used to automate feature extraction and analysis
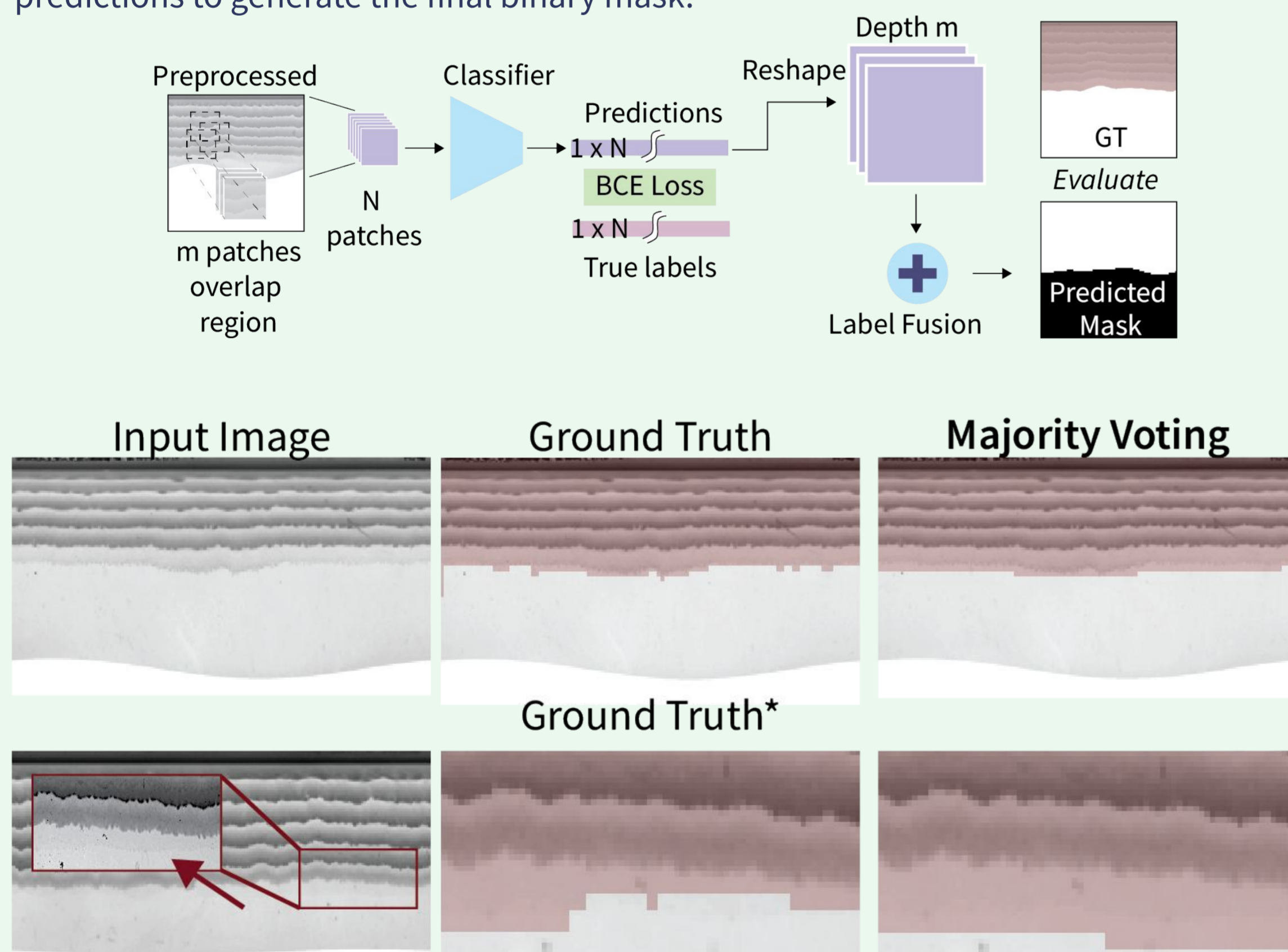
Overview of Project

1 Dataset of Images → Preprocessing Methods → Segmentation models → Output Masks 2a / 2b → 3 Measure Colony Features

1. Dataset & Preprocessing

	Swarm Assay	Scanner Settings	Segmentation	Dataset Size	Description	Model Input Type
	1.3-1.5% agar, 30 min plate drying, 2 uL OD 0.1 culture inoculated, 15 min drying, 24 hour growth at 25-37ºC	400 DPI, 24-bit color, Orientation: agar facing ceiling, remove plate lid	Colony	306	Colonies covering part or full plate, agar-only images	Image patches (150,000 positive/112,000 negative)
			Ring Boundaries	558 (Cycle 1), 300 (Cycle 2)	Colonies with distinct ring boundaries	Fullsize images padded (border reflect method)

Preprocessing: Input → Petri rim removal → Thresholding for inoculum → Correct center selection → Polar transform → Preprocessed for colony seg. → Preprocessed for ring seg.
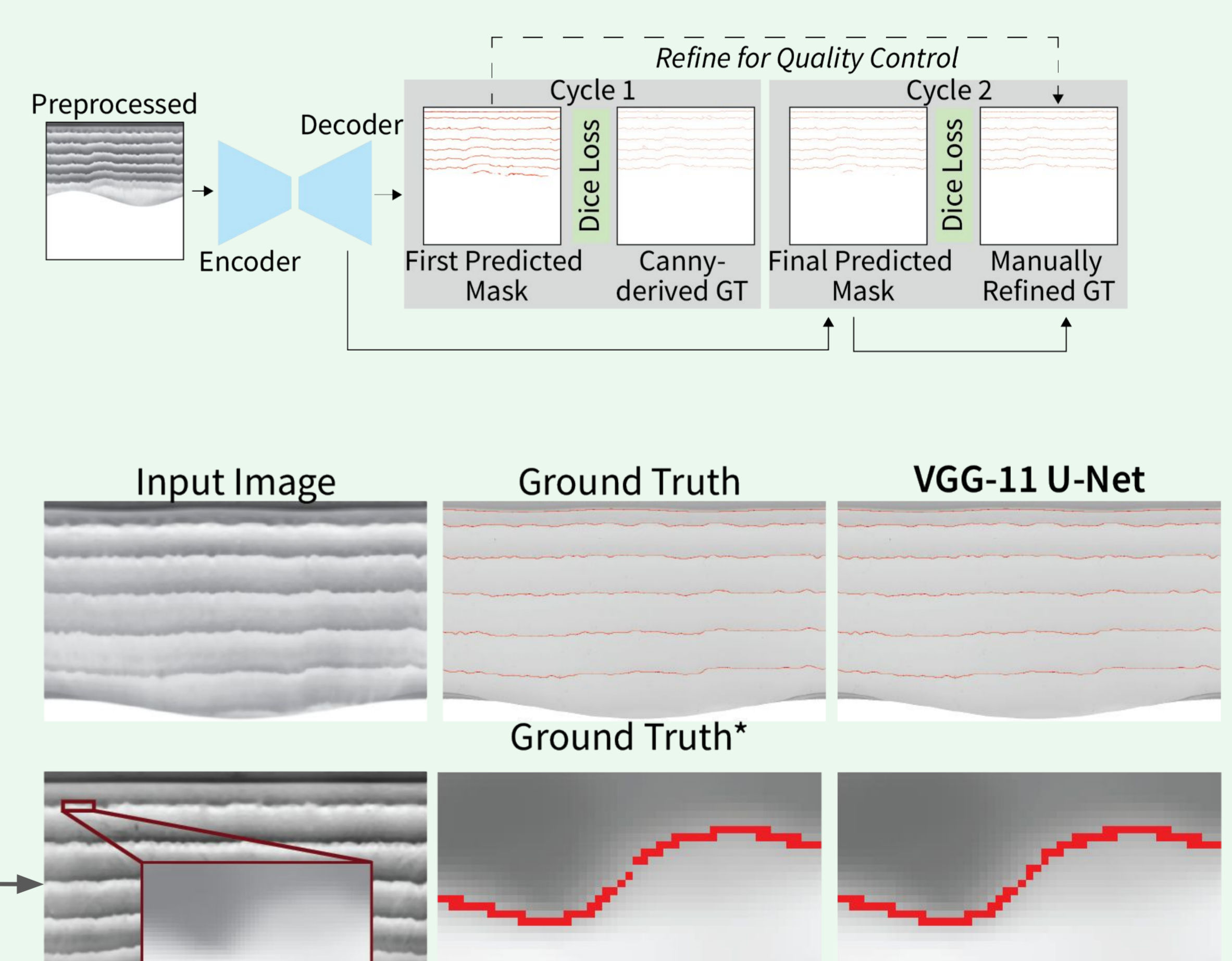
2a. Colony Segmentation

For colony segmentation, a CNN was trained to classify overlapping patches of a given image as colony or background. Majority voting fused the overlapping predictions to generate the final binary mask.

Preprocessed → m patches overlap region → N patches → Classifier → Predictions $1 \times N$ → BCE Loss / True labels → Reshape → Depth m → Label Fusion → GT / Evaluate → Predicted Mask

Input Image — Ground Truth — Majority Voting

Ground Truth*

*In some cases, the models even improved on the ground truth predictions.
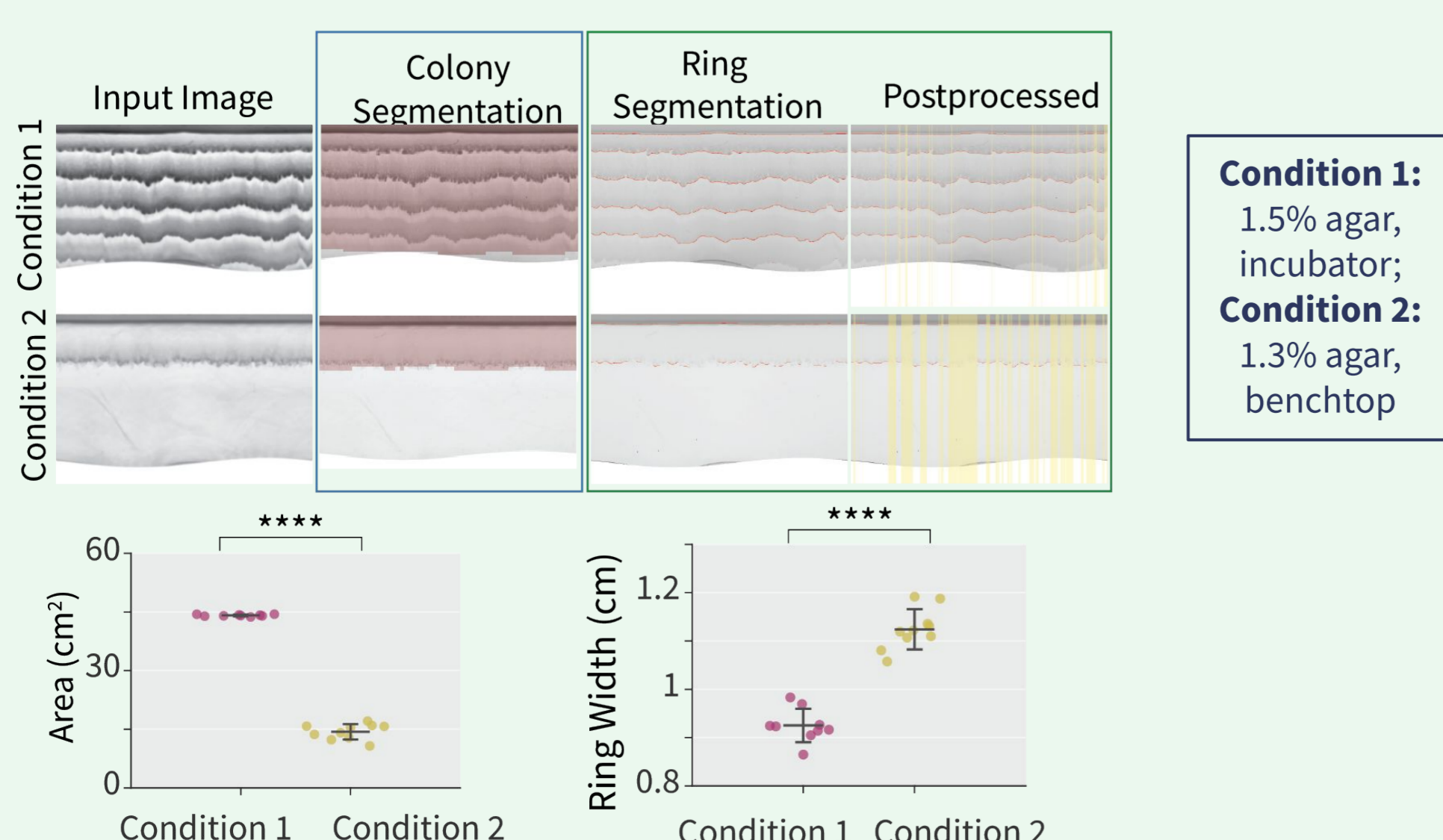
2b. Ring Boundary Segmentation

Ring boundary segmentation training was done with an iterative process; first, Canny-derived ground truths were used. The trained model generated predictions on a larger set of images, including previously unseen ones & more complex patterns. Some of these predictions were manually refined, creating a more diverse set of ground truths for the second round. After retraining the model from scratch on these, the final predictions underwent basic post-processing for noise reduction.

Preprocessed → Encoder → Decoder → *Refine for Quality Control* → Cycle 1: First Predicted Mask / Dice Loss / Canny-derived GT → Cycle 2: Final Predicted Mask / Dice Loss / Manually Refined GT

Input Image — Ground Truth — VGG-11 U-Net

Ground Truth*

3. Application

We generated images of two standard types of colony patterns (9 each) and applied our segmentation pipeline. The resulting masks enabled calculation of colony area and ring width, easily performed on all images at once.

Input Image — Colony Segmentation — Ring Segmentation — Postprocessed

Condition 1: 1.5% agar, incubator;
Condition 2: 1.3% agar, benchtop

Area (cm²): Condition 1 vs Condition 2 (****)
Ring Width (cm): Condition 1 vs Condition 2 (****)

Model Performance

Label Fusion Method	Accuracy	Precision	Recall	Dice	IoU
Single convolutional layer	0.9573	0.9579	0.8708	0.6679	0.9787
Averaging Output	0.9822	0.9518	**0.9749**	0.9320	0.9904
Majority Voting	**0.9841**	**0.9624**	0.9724	**0.9328**	**0.9912**

U-Net Encoder	Decoder attention	Training data augmentation	Accuracy	Precision	Recall	Dice	IoU
VGG-11	None	Yes	0.9969	0.7452	0.7998	0.7487	0.6458
	None	No	**0.9982**	**0.8789**	**0.8117**	**0.8324**	**0.7585**
	SCSE	No	0.9980	0.8635	0.8059	0.8200	0.7430
VGG-11 + batchnorm	None	No	0.9980	0.8622	0.8055	0.8166	0.7439
	SCSE	No	0.9980	0.8603	0.8102	0.8223	0.7446
ResNet18	None	No	0.9978	0.8432	0.8004	0.7959	0.7253
	SCSE	No	0.9980	0.8629	0.8044	0.8207	0.7394
EfficientNet-B0	None	No	0.9977	0.8337	0.7974	0.7822	0.7132
	SCSE	No	0.9976	0.8436	0.7741	0.7886	0.7004

Acknowledgements

References

1. Yuang et al., *Urol Int* 2021, 105:354-61.
2. Schaffer & Pearson, *Microbiol Spectr* 2015, 3.5.10.
3. Armbruster et al., J Bacteriol 2013, 195(6):1305-19.
4. Rather, *Environ Microbiol* 2005, 7(8):1065-73.