

ReScale4DL: Balancing Pixel and Contextual
Information for Enhanced Bioimage
Segmentation
Supplementary Notes

May 2, 2025

Supplementary Note 1: Deep learning model training hyperparameters

<i>C. elegans</i>		<i>E. coli</i>		<i>S. aureus</i>	
Network	2D U-Net	Network	2D U-Net	Network	2D StarDist
Epochs	1000	Epochs	1000	Epochs	1000
Patch size (per resizing factor)	512 ($\times 2^{-2, -1, 0}$) 256 ($\times 2$) 128 ($\times 2^2$) 64 ($\times 2^3$) 32 ($\times 2^4$)	Patch size (per resizing factor)	512 ($\times 2^{-2, -1, 0}$) 256 ($\times 2$) 128 ($\times 2^2$)	Patch size (per resizing factor)	2048 ($\times 2^{-2, -1, 0}$) 1184 ($\times 2$) 576 ($\times 2^2$) 272 ($\times 2^3$) 128 ($\times 2^4$)
Batch size	5	Batch size	5	Batch size	2
Validation [%]	10	Validation [%]	10	Validation [%]	10
Learning rate	0.0001	Learning rate	0.0001	Learning rate	0.0003
Pooling steps	2	Pooling steps	2	Grid parameter	2
Minimal fraction	0.05	Minimal fraction	0.05	Number of rays	32

Table 1. The network architecture and the training parameters for each dataset were preserved. The patch size for the input during training was dynamically changed with the image resizing to maintain the same field of view, except for the upsampling due to limited GPU capabilities. The values $2^{-2, -1}$ correspond to image upsamplings, 2^0 is the original image resolution and $2^{1, \dots, 4}$ correspond to image downsampling.

Supplementary Note 2: Optimising the pixel size for foundation models

Here we provide a visual example of segmentation performance using foundation models for images with different pixel sizes. We performed instance segmentation of *Escherichia coli* (*E. coli*), *Caenorhabditis elegans* (*C. elegans*), and *Staphylococcus aureus* (*S. aureus*) samples using MicroSAM (23) software, with the same rescaling factors used for the Figs. 2 and 4. For simplicity, we used their 2D Annotator plugin in Napari to deploy interactive segmentation with a single-point prompt-based approach, followed by the automatic instance segmentation. Rescaled images were individually segmented by manually pointing to the same structural landmark each time. For all experiments, we chose the Vision Transformer (ViT) Large backbone architecture, a fine-tuned version of the original Segment Anything Model (SAM) (26) for cellular and nuclear segmentation in light microscopy (ViT-l-1m). Importantly, the segmentation results provided here may not be optimal and only aim to show the variability of the model according to the image resolution under the same conditions. Indeed, the results could be improved by changing the prompt or by increasing their number.

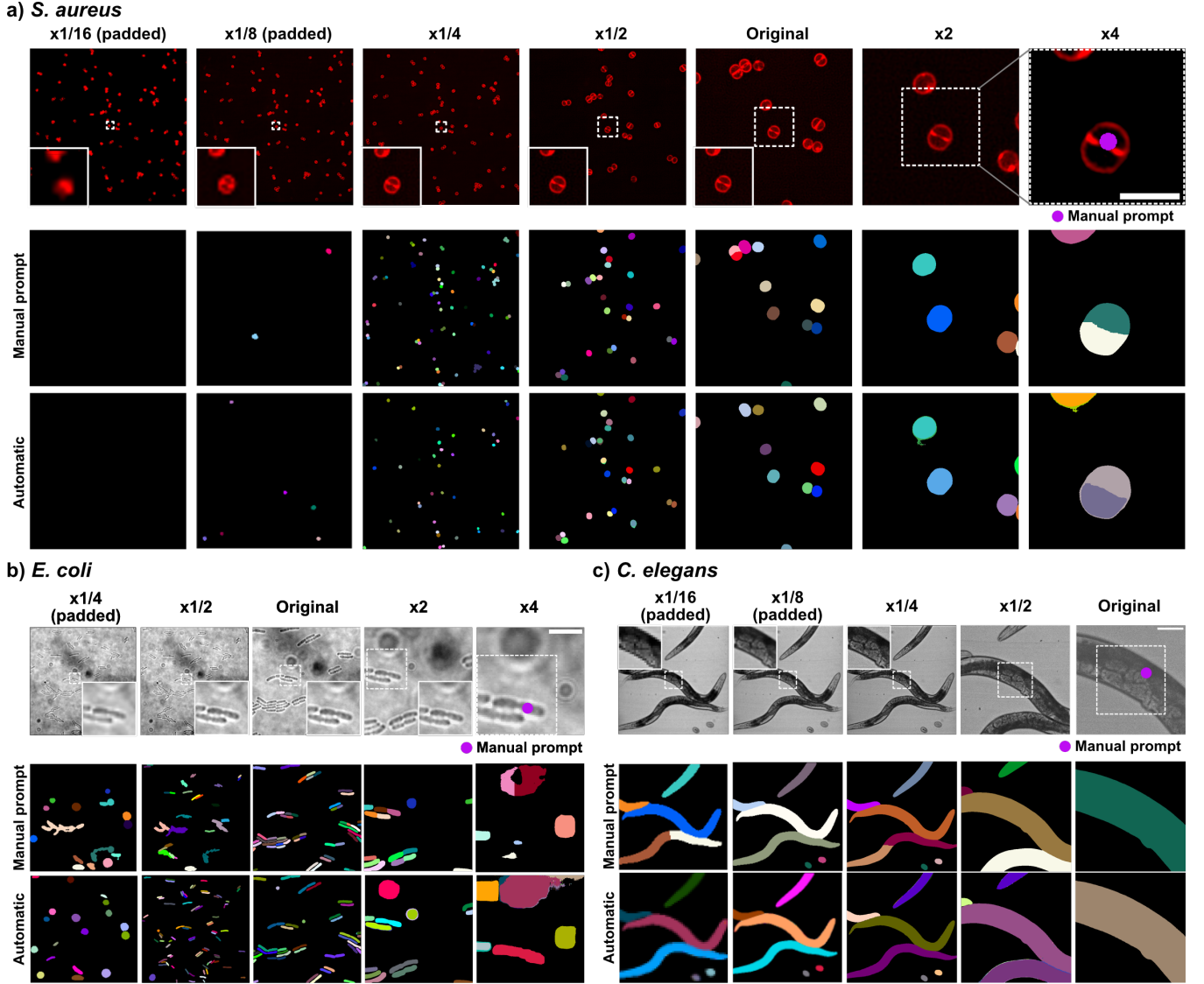


Fig. 5. Segmentation results of Segment Anything for Microscopy on images with different pixel sizes using a single point prompting. MicroSAM (23) was used to obtain the instance segmentation of a) *S. aureus*, b) *E. coli* and c) *C. elegans* example images. For simplicity, we followed a single point-wise prompting strategy, pointing to the same object on each rescaled image (*i.e.*, the manual prompt was the same on each panel), and run the automatic instance segmentation pipeline. All the images matched model input dimensions (512×512 pixels) to avoid internal tiling or image re-scaling. The images that had smaller dimensions were padded with zeros. Scale bars correspond to $25 \mu m$ for a), and $50 \mu m$ for b-c). Square boxes correspond to the same physical area on each image.

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