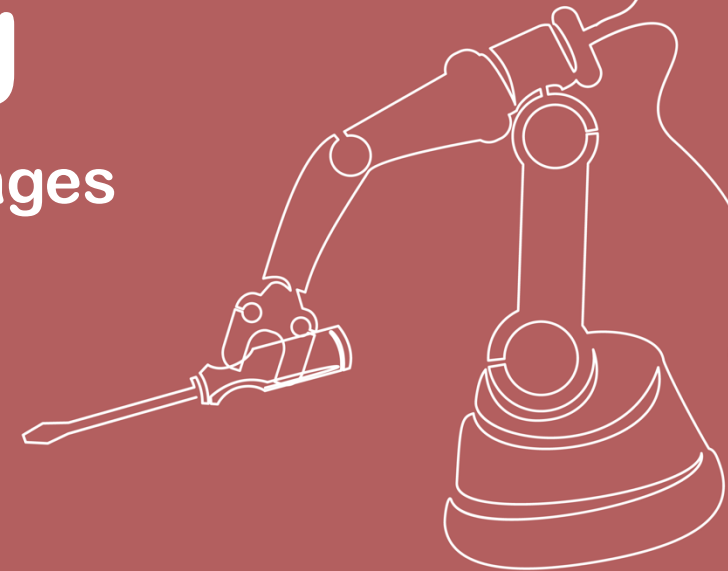


Molecular Imaging

Automated Analysis of Microscopic Images

28.10.24



Overview

- Cell Segementaion/Detection
- SOTA Models in cell segmentation
 - MEDIAR
 - Microsam
- Tools
 - Fiji + Plugins
 - Napari + Plugins

Course schedule

SW	CW	Date	Topic	Lecturer
1	38	16.09.24	Introduction	Steffi Lehmann, Céline Reinbold
2	39	23.09.24	Nuclear Imaging	Robert Vorburger
3	40	30.09.24	In-Vivo MR Spectroscopy	Andi Hock
4	41	07.10.24	In-Vivo MR Spectroscopy	Andi Hock
5	42	14.10.24	Optical imaging	Steffi Lehmann, Besmira Sabani
6	43	21.10.24	Optical imaging	Steffi Lehmann, Besmira Sabani
7	44	28.10.24	Automated analysis of microscopic images – introduction	Stefan Glüge
8	45	04.11.24	Automated analysis of microscopic images – group work	Stefan Glüge
9	46	11.11.24	Automated analysis of microscopic images – group work	Stefan Glüge
10	47	18.11.24	Automated analysis of microscopic images – group work	Stefan Glüge
11	48	25.11.24	Automated analysis of microscopic images - presentations	Stefan Glüge, Steffi Lehmann, Besmira Sabani
12	49	02.12.24	Drug Development	Steffi Lehmann, Besmira Sabani
13	50	09.12.24	Imaging in tumor and cancer diseases	Steffi Lehmann, Besmira Sabani
14	51	16.12.24	Recap	Steffi Lehmann, Besmira Sabani



Assessments

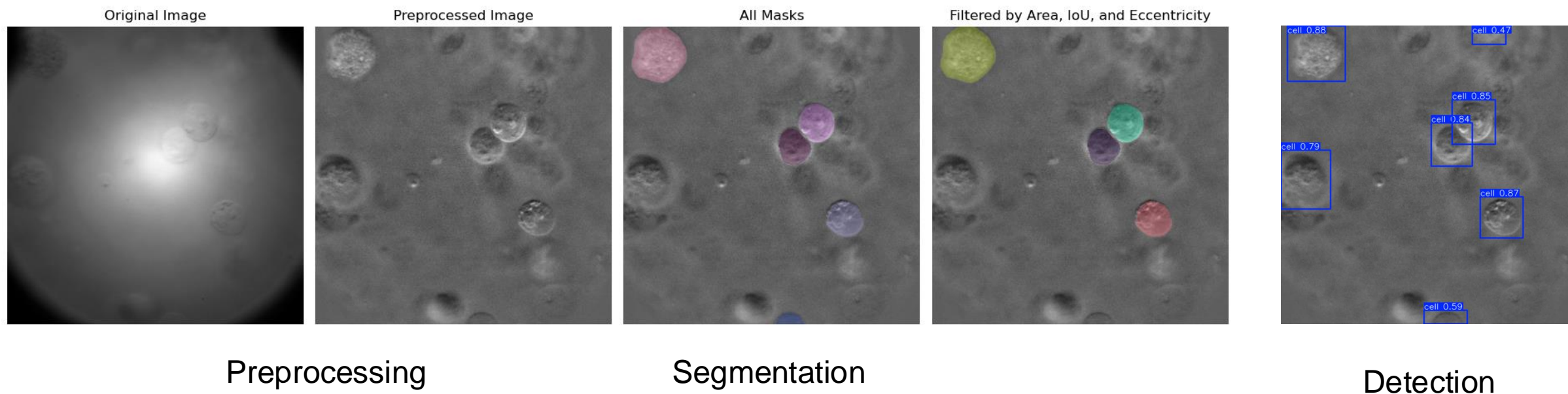
- Course work during the semester: 50%
 - Start: 28. October 2024
 - Presentations: 25.11.2024
- Oral exam at the end of the semester: 50%

Contact Information

- Céline Reinbold: general questions (renb@zhaw.ch)
- Robert Vorburger (voru@zhaw.ch), Andi Hock (hocn@zhaw.ch), Steffi Lehmann (leht@zhaw.ch), Stefan Glüge (glue@zhaw.ch)

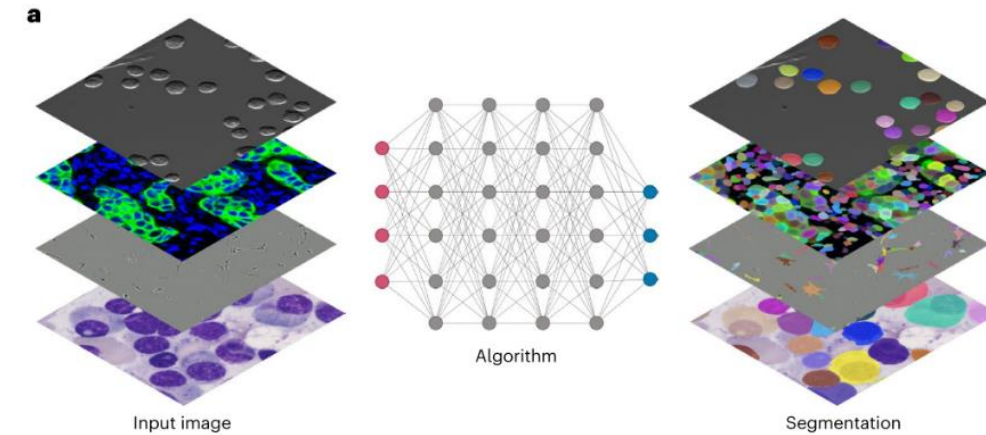
Cell Segmentation/Detection

- Cell segmentation is a fundamental task for computational biology analysis
- Task: identifying cell instances in a given image
- Often first step in various downstream tasks



Cell Segmentation – State of the art

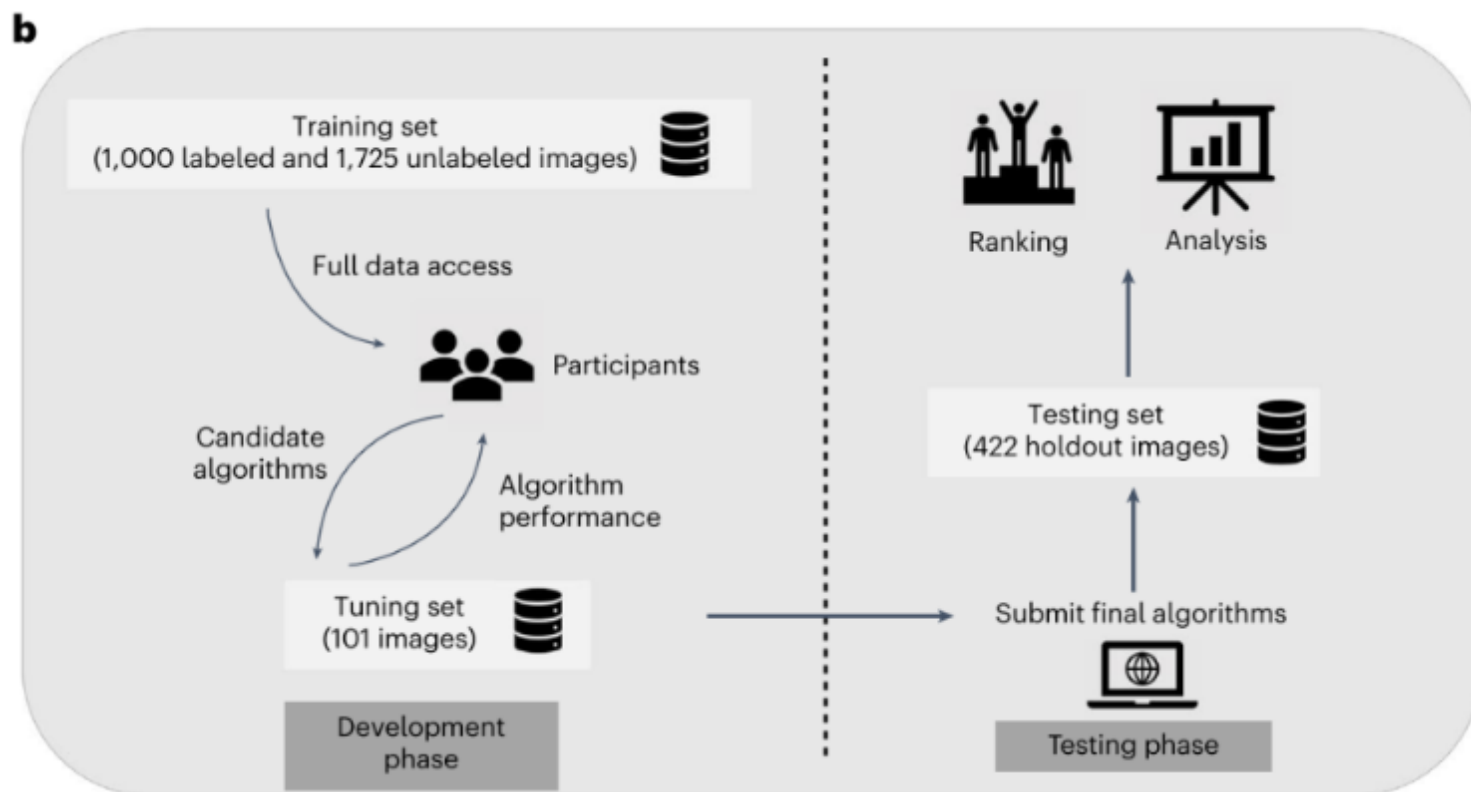
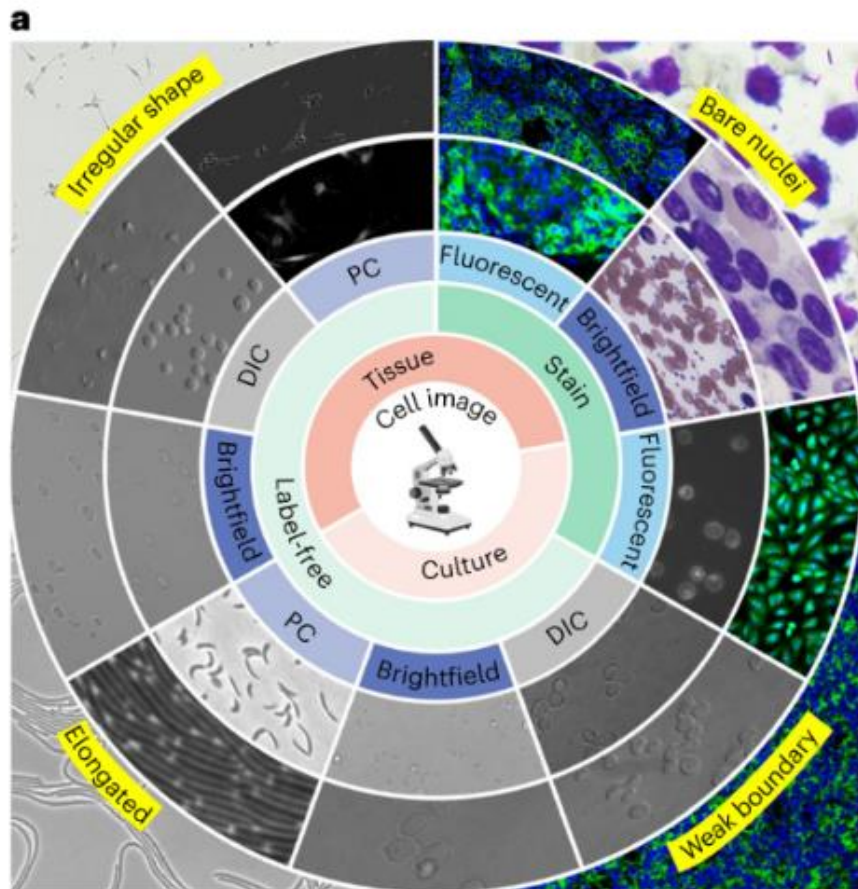
- Cell segmentation methods are (were) tailored to specific modalities or require manual interventions to specify hyperparameters in different experimental settings
- Biomedical image data science competitions accelerated the development of algorithms
- Microscopy image analysis comps, Cell Tracking Challenge (2017), Data Science Bowl Challenge (2018), Colon Nuclei Identification and Counting (CoNIC) Challenge (2022) -> *focused on a limited subset of microscopy image types*
- The multimodality cell segmentation challenge (2022) NeurIPS -> multimodality cell segmentation benchmark, comprised of **>1500** labeled images derived **>50** diverse biological experiments



Ma, J., Xie, R., Ayyadhury, S., Ge, C., Gupta, A., Gupta, R., Gu, S., Zhang, Y., Lee, G., Kim, J., Lou, W., Li, H., Upschulte, E., Dickscheid, T., de Almeida, J. G., Wang, Y., Han, L., Yang, X., Labagnara, M., ... Wang, B. (2024). The multimodality cell segmentation challenge: toward universal solutions. *Nature Methods*. <https://doi.org/10.1038/s41592-024-02233-6>

<https://neurips22-cellseg.grand-challenge.org>

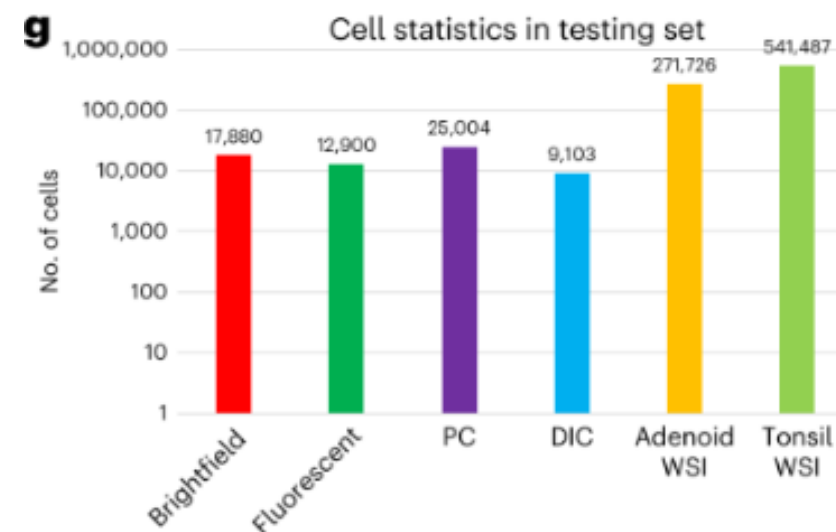
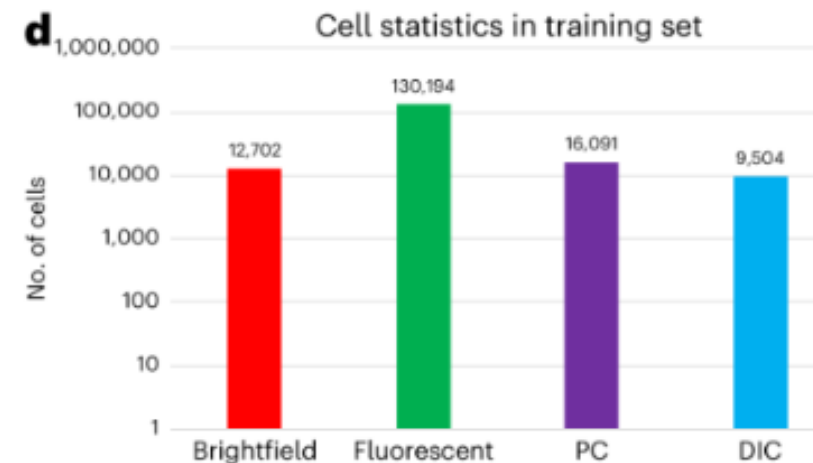
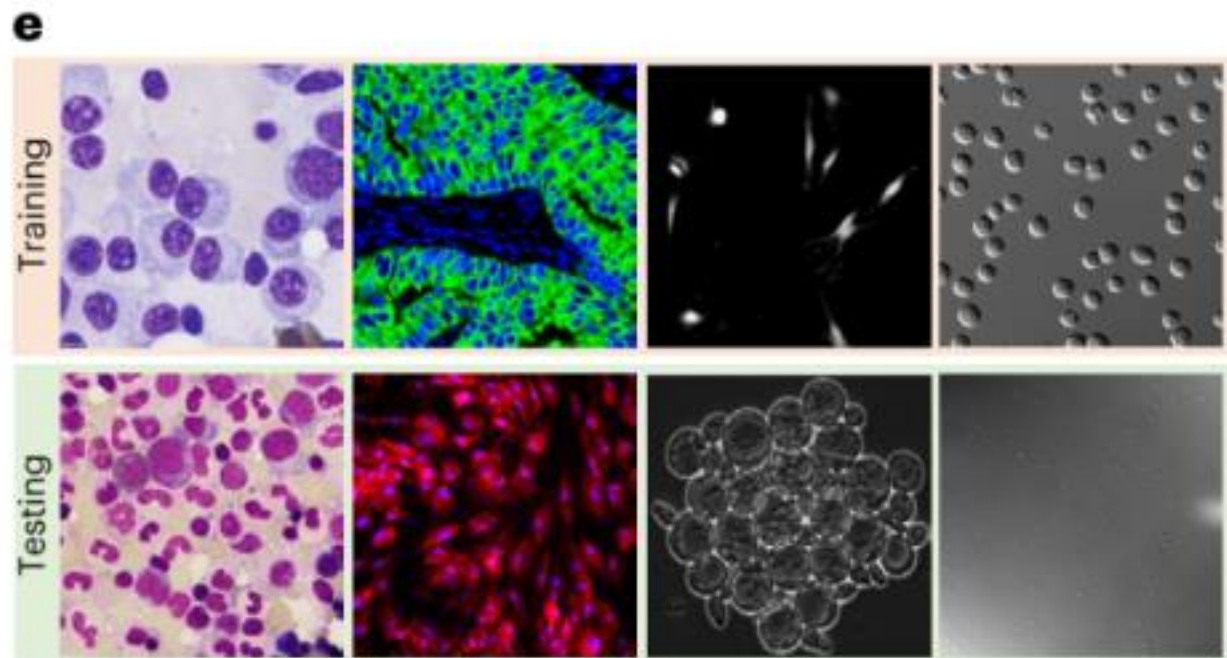
Multimodality cell segmentation challenge (2022)



Ma, J., Xie, R., Ayyadhury, S., Ge, C., Gupta, A., Gupta, R., Gu, S., Zhang, Y., Lee, G., Kim, J., Lou, W., Li, H., Upschulte, E., Dickscheid, T., de Almeida, J. G., Wang, Y., Han, L., Yang, X., Labagnara, M., ... Wang, B. (2024). The multimodality cell segmentation challenge: toward universal solutions. *Nature Methods*. <https://doi.org/10.1038/s41592-024-02233-6>

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<https://neurips22-cellseg-grand-challenge.org>

Multimodality cell segmentation challenge (2022) - Algorithms

- *All* algorithms in this challenge employed deep-learning-based approaches
- Algorithms predominantly relied on convolutional neural networks (CNNs) such as U-Net and DeepLab
- CNN-based cell segmentation models exhibited limited generalization capability on diverse images without additional human intervention (manual selection of channels and/or fine-tuning)
- Transformers integrating attention mechanisms have exhibited robust performance and generalization capabilities across various computer vision tasks and achieved notably superior performance

Multimodality cell segmentation challenge (2022) - Algorithms

Table 1 | Characteristics of the top three best-performing algorithms in preprocessing, data augmentation, network architecture and post-processing

Team	Preprocessing		Data augmentation				Network architecture		Post-processing
	IN	PS	IS	ED	UD	Others	Encoder backbone	Decoder heads	
T1-osilab ²⁵	✓	✓	✓	✓	–	Cell-wise intensity perturbation; boundary exclusion; oversample minor modality	SegFormer	Cell probability head; gradient fields regression head	Gradient tracking; exclude small cells; fill holes; TTA;
T2-sribdmed ²⁹	✓	✓	✓	✓	✓	–	ConvNeXt	Cell probability head; radial distance head; gradient fields regression head	NMS; watershed
T3-cells ³¹	✓	✓	✓	✓	✓	Cell-aware rescaling	ResNeXt-101	Classification head; contour regression head; local refinement regression head; boundary uncertainty estimation head	NMS; convert contours to masks; region growing

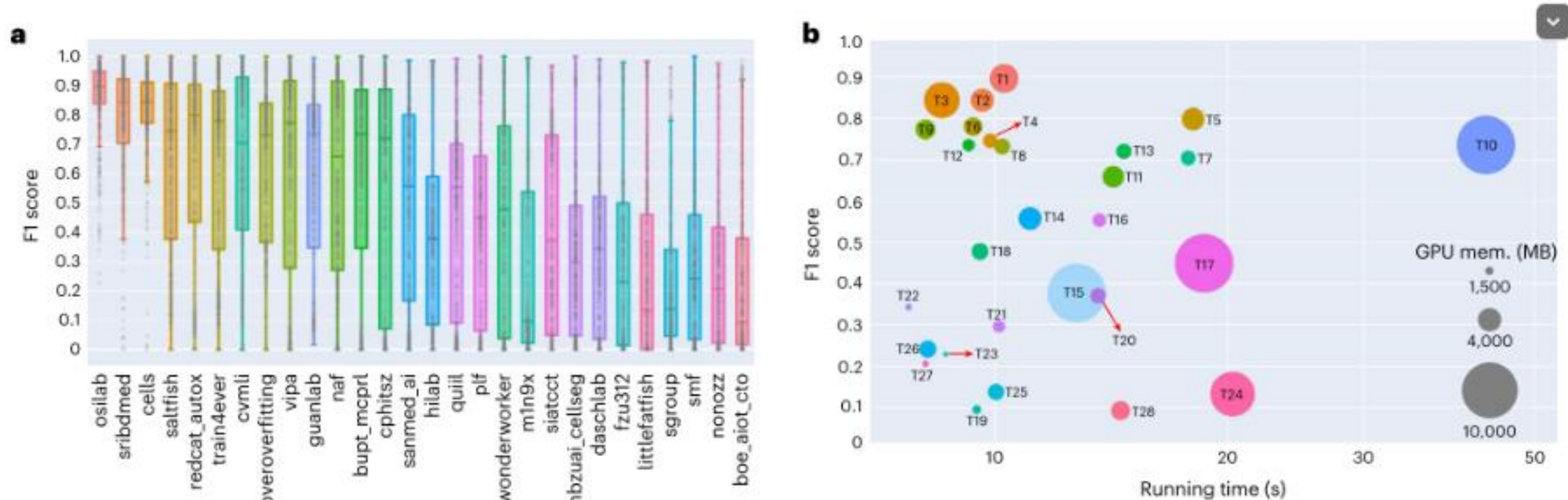
IN, intensity normalization; PS, patch sampling; IS, intensity and spatial data augmentation; ED, external dataset; UD, unlabeled data; NMS, nonmaximum suppression; TTA, test-time augmentation. – indicates not used.

Ma, J., Xie, R., Ayyadhury, S., Ge, C., Gupta, A., Gupta, R., Gu, S., Zhang, Y., Lee, G., Kim, J., Lou, W., Li, H., Upschulte, E., Dickscheid, T., de Almeida, J. G., Wang, Y., Han, L., Yang, X., Labagnara, M., ... Wang, B. (2024). The multimodality cell segmentation challenge: toward universal solutions. *Nature Methods*.

<https://doi.org/10.1038/s41592-024-02233-6>

<https://neurips22-cellseg.grand-challenge.org>

Multimodality cell segmentation challenge (2022) - Results



Ma, J., Xie, R., Ayyadhury, S., Ge, C., Gupta, A., Gupta, R., Gu, S., Zhang, Y., Lee, G., Kim, J., Lou, W., Li, H., Upschulte, E., Dickscheid, T., de Almeida, J. G., Wang, Y., Han, L., Yang, X., Labagnara, M., ... Wang, B. (2024). The multimodality cell segmentation challenge: toward universal solutions. *Nature Methods*.

<https://doi.org/10.1038/s41592-024-02233-6>

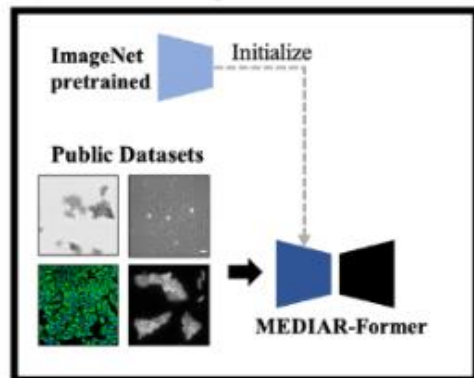
<https://neurips22-cellseg.grand-challenge.org>

MEDIAR: Harmony of Data-Centric and Model-Centric for Multi-Modality Microscopy

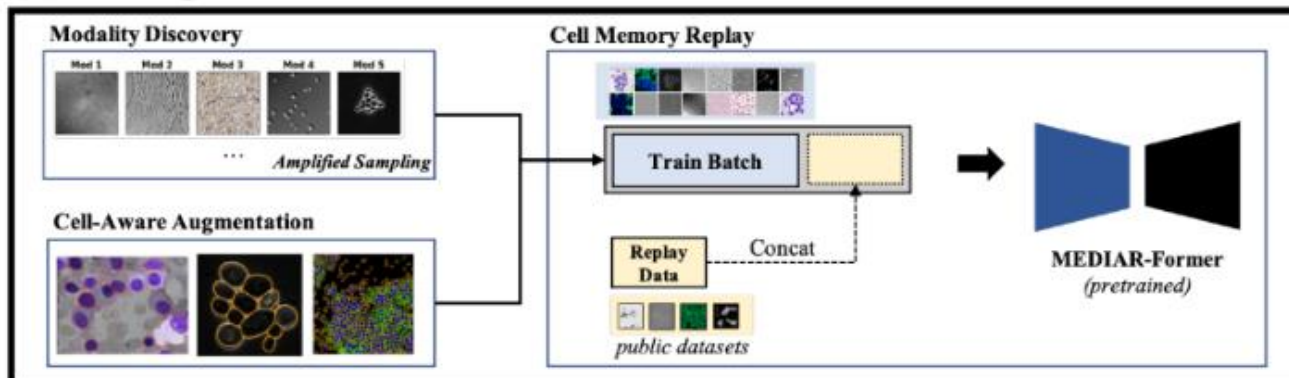
- Framework to conduct cell segmentation under multi-modality by a single generalist model
- Combining data-centric and model-centric approaches
- Data-Centric: applied learning strategy to balance the latent modalities and retain the knowledge from pretraining
- Model-Centric: provide a model structure to capture cell instances and a corresponding inference strategy to conduct prediction for high-resolution images under limited time budget
- *Released the trained model*, which achieved the F1-score 0.9067 on multi-modality microscopy challenge datasets

MEDIAR Overview

(a) Pretraining



(b) Training



(c) Cell Segmentation Inference

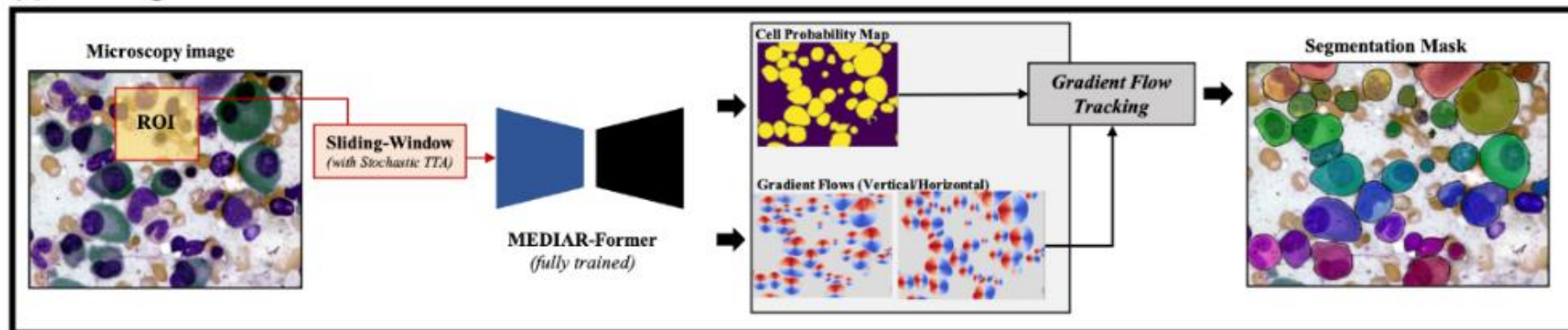
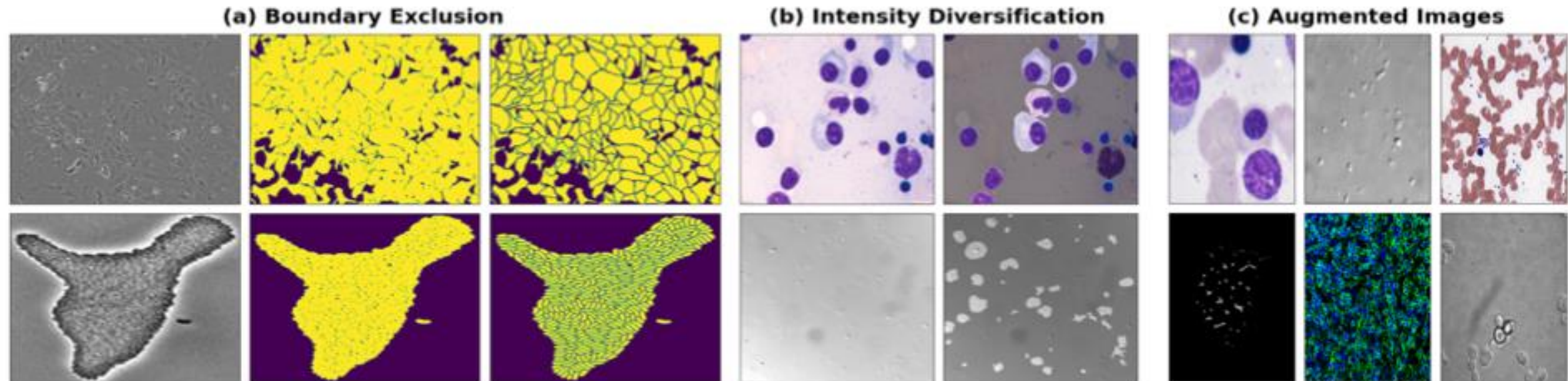


Figure 3: An overview of MEDIAR framework.

Lee, G., Kim, S., Kim, J., & Yun, S.-Y. (2023). MEDIAR: Harmony of Data-Centric and Model-Centric for Multi-Modality Microscopy. *Proceedings of The Cell Segmentation Challenge in Multi-Modality High-Resolution Microscopy Images*, PMLR, 1–16. <https://github.com/Lee-Gihun/MEDIAR>

MEDIAR Cell-Aware Augmentation

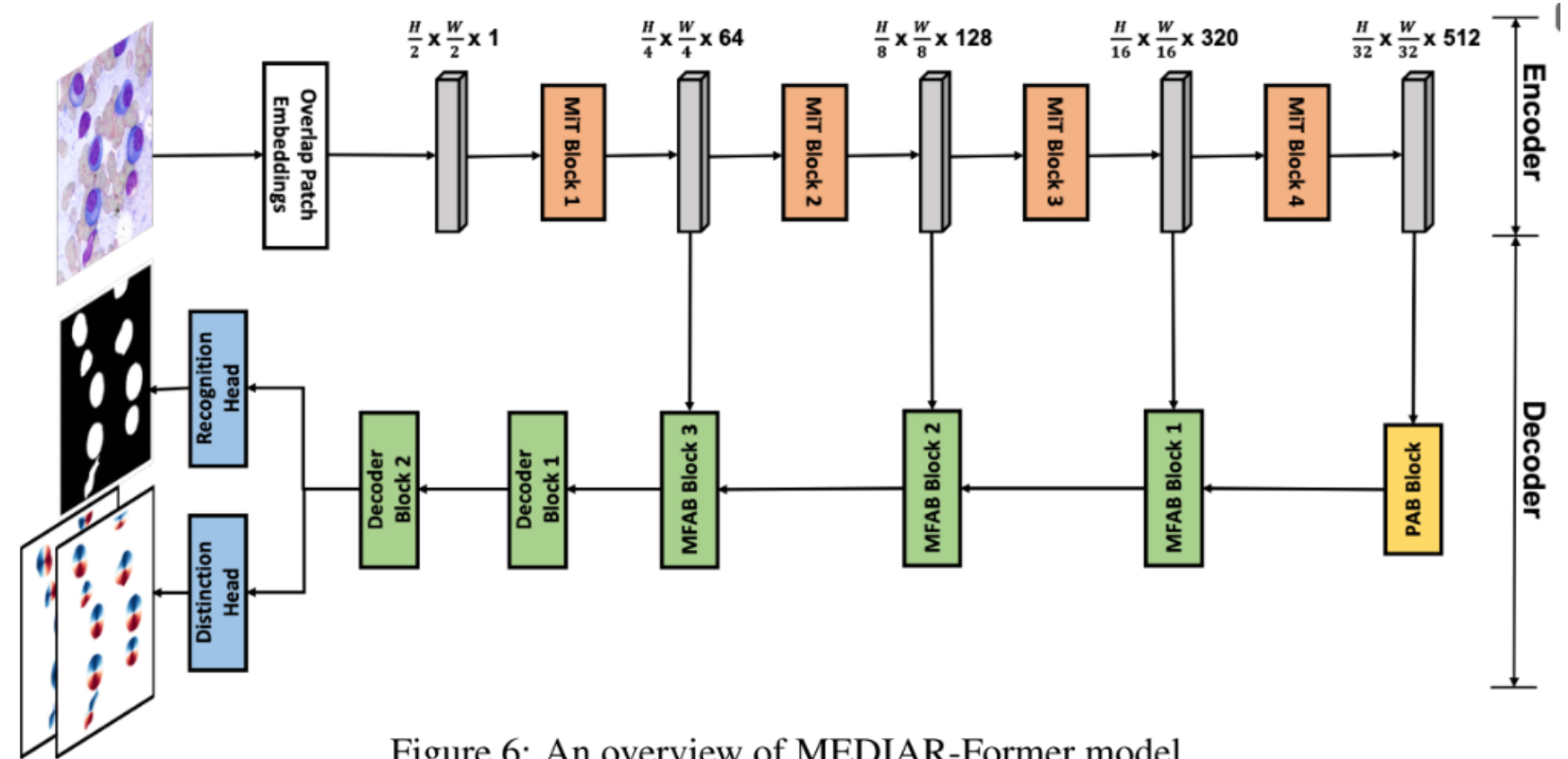
- Used intensive combination of data augmentation strategies
- Two cell-aware augmentations to improve generalization
 - Intensity Diversification – randomize intensity cell-wise
 - Exclude boundary pixels in the label (only in pre-training)



Lee, G., Kim, S., Kim, J., & Yun, S.-Y. (2023). MEDIAR: Harmony of Data-Centric and Model-Centric for Multi-Modality Microscopy. *Proceedings of The Cell Segmentation Challenge in Multi-Modality High-Resolution Microscopy Images*, PMLR, 1–16. <https://github.com/Lee-Gihun/MEDIAR>

MEDIAR Overview

- MEDIAR-Former follows the U-Net design
- MEDIAR-Former uses two separate heads:
 - Cell Recognition (CR)
 - Cell Distinction (CD)



Lee, G., Kim, S., Kim, J., & Yun, S.-Y. (2023). MEDIAR: Harmony of Data-Centric and Model-Centric for Multi-Modality Microscopy. *Proceedings of The Cell Segmentation Challenge in Multi-Modality High-Resolution Microscopy Images*, PMLR, 1–16. <https://github.com/Lee-Gihun/MEDIAR>

MEDIAR – Cell Instance Segmentation

- After filtering cell candidates, all pixels are aggregated into the cell indices by iteratively following the spatial gradient fields
- MEDIAR conducts gradient flow tracking in a non-overlapped sliding window manner with patch size 2000 x 2000.

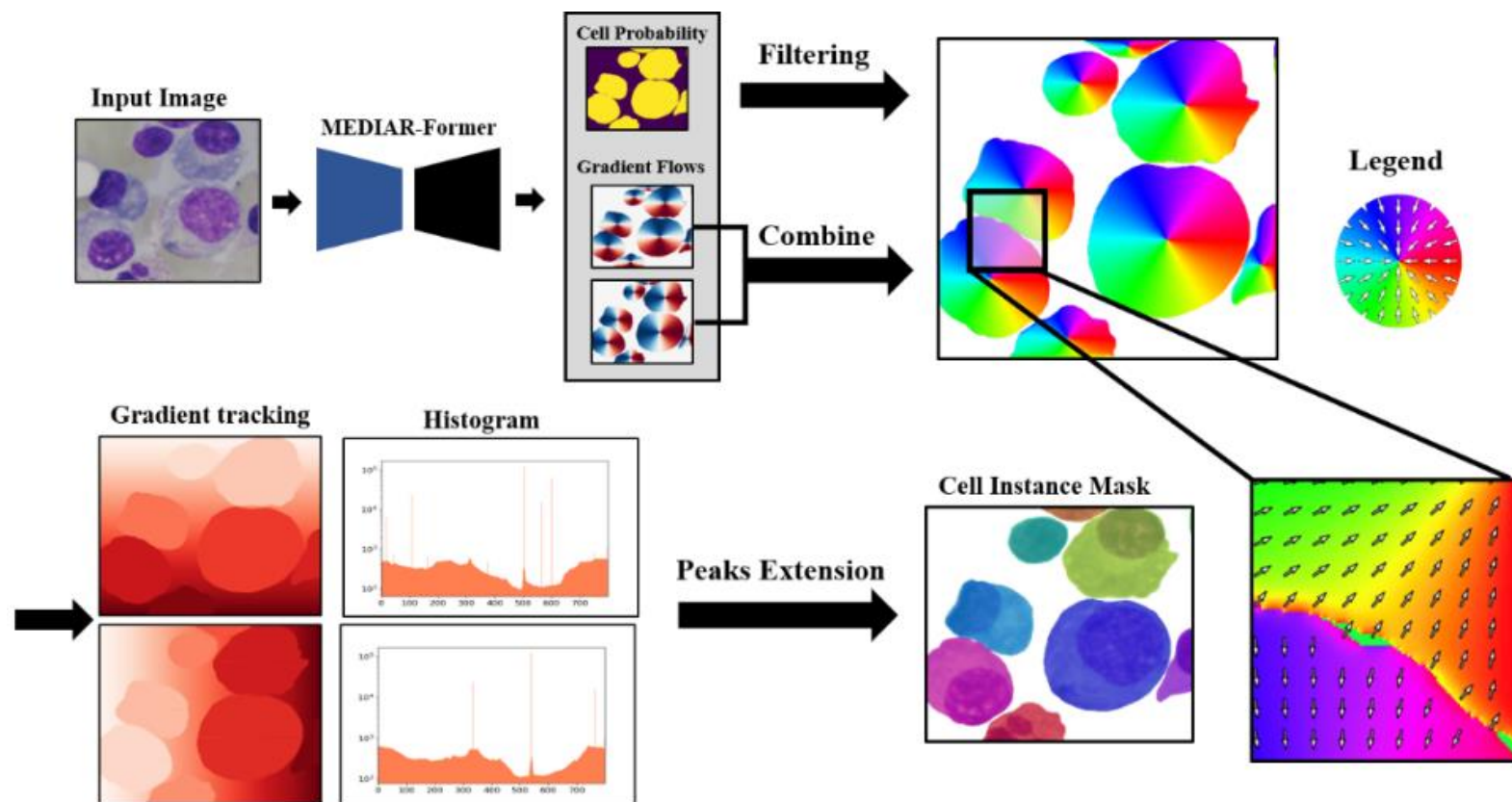


Figure 8: An example of gradient flow tracking for cell instance segmentation.

Lee, G., Kim, S., Kim, J., & Yun, S.-Y. (2023). MEDIAR: Harmony of Data-Centric and Model-Centric for Multi-Modality Microscopy. *Proceedings of The Cell Segmentation Challenge in Multi-Modality High-Resolution Microscopy Images*, PMLR, 1–16. <https://github.com/Lee-Gihun/MEDIAR>

MEDIAR – Failure Cases

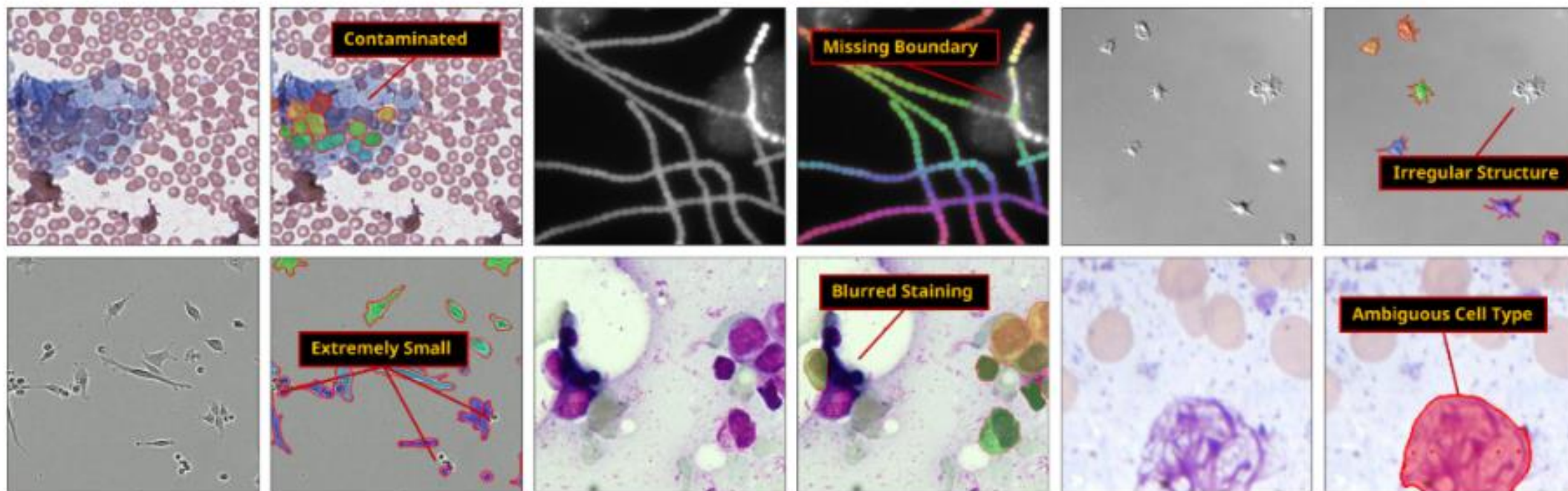
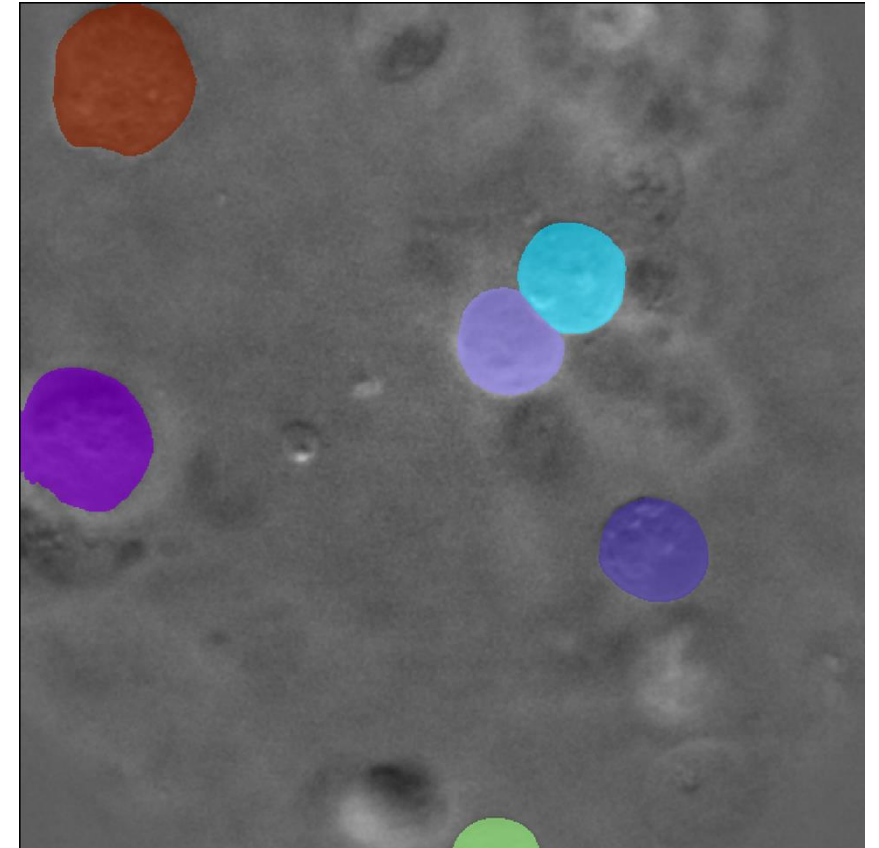
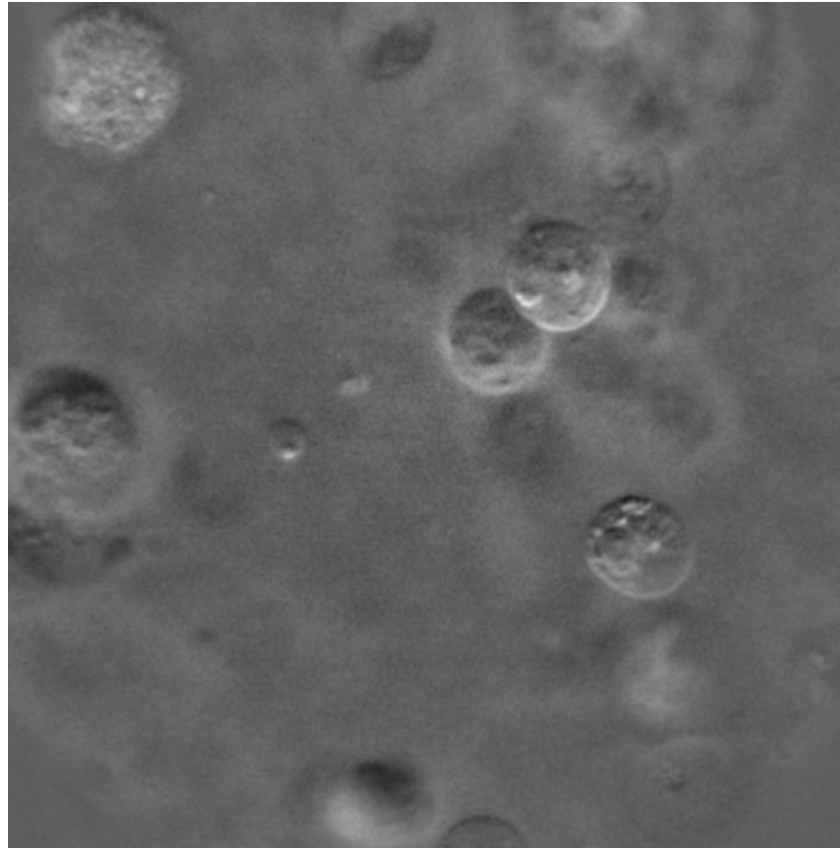


Figure 13: MEDIAR failure cases across different modalities. We zoomed in on the images for clear visualization and categorized the failure types. Note that the magnifications are different.

Lee, G., Kim, S., Kim, J., & Yun, S.-Y. (2023). MEDIAR: Harmony of Data-Centric and Model-Centric for Multi-Modality Microscopy. *Proceedings of The Cell Segmentation Challenge in Multi-Modality High-Resolution Microscopy Images*, PMLR, 1–16. <https://github.com/Lee-Gihun/MEDIAR>

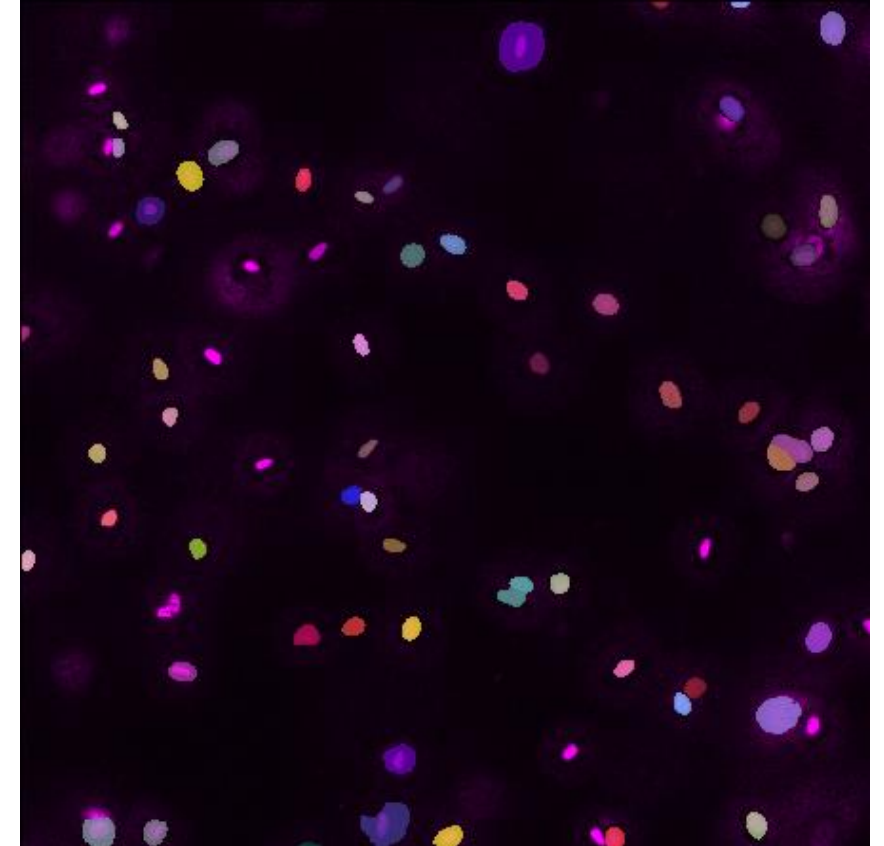
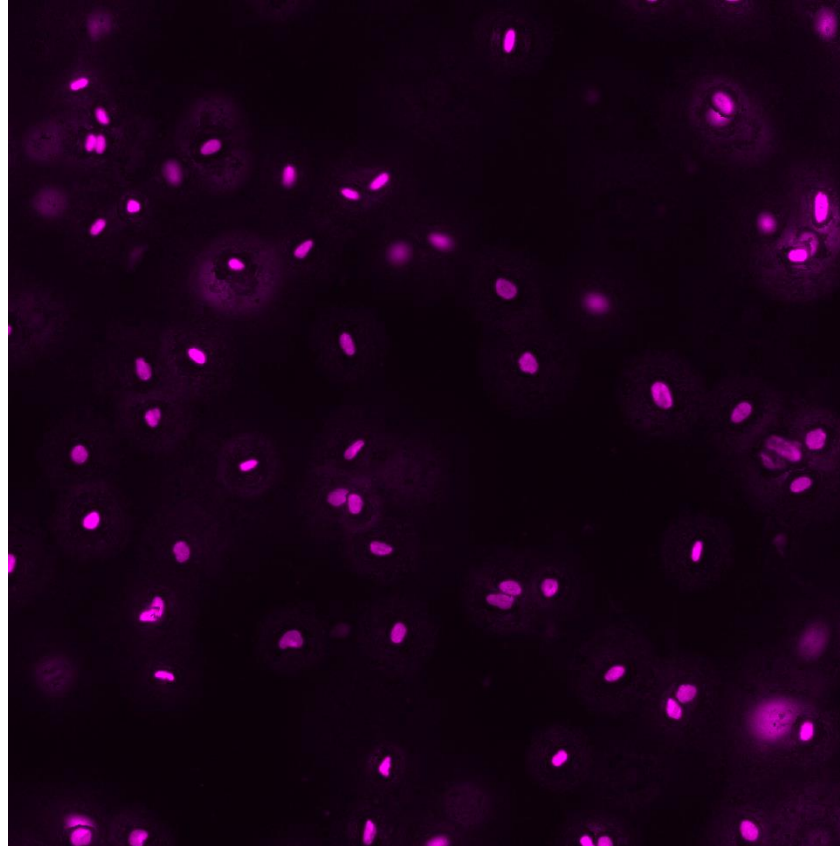
MEDIAR – CHO cell segmentation

- Runtime on M3 pro: approx. 60s



MEDIAR – Cell segmentation in 3D culture

- Runtime on M3 pro: approx. 20min



Segment Anything for Microscopy

- Introduces a tool for *interactive* and automatic segmentation
- Track objects in multi-dimensional microscopy data
- Based on Segment Anything vision foundation model (MetaAI)
- Training specialized models tailored for microscopy data
- Provide annotation tools for interactive volumetric segmentation and tracking

Segment Anything for Microscopy

Anwai Archit, Sushmita Nair, Nabeel Khalid, Paul Hilt, Vikas Rajashekar, Marei Freitag, Sagnik Gupta, Andreas Dengel, Sheraz Ahmed, Constantin Pape

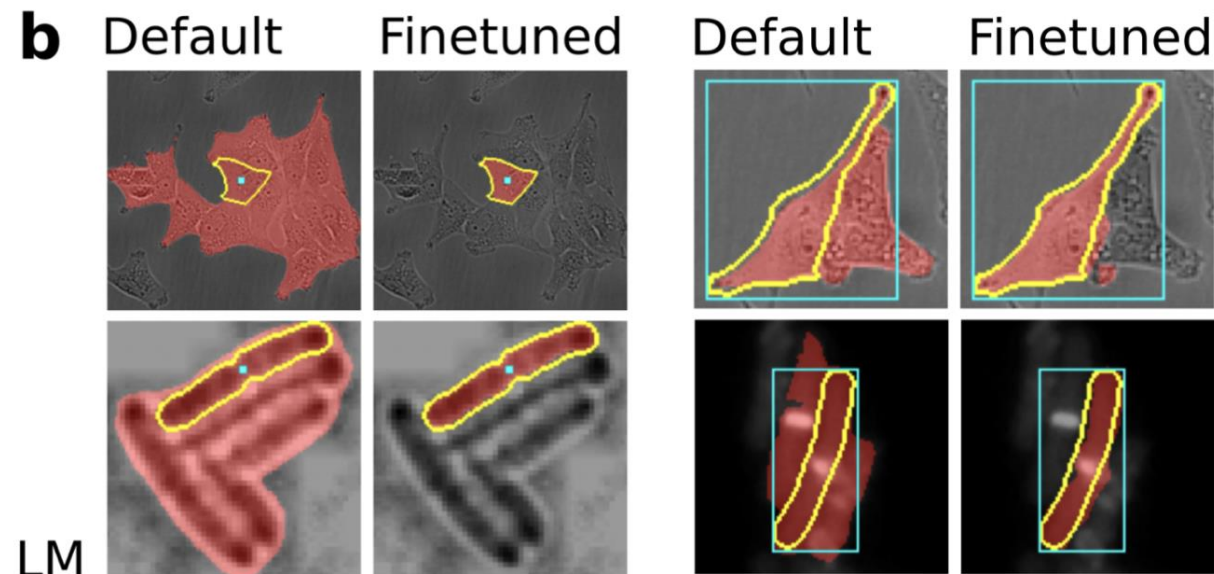
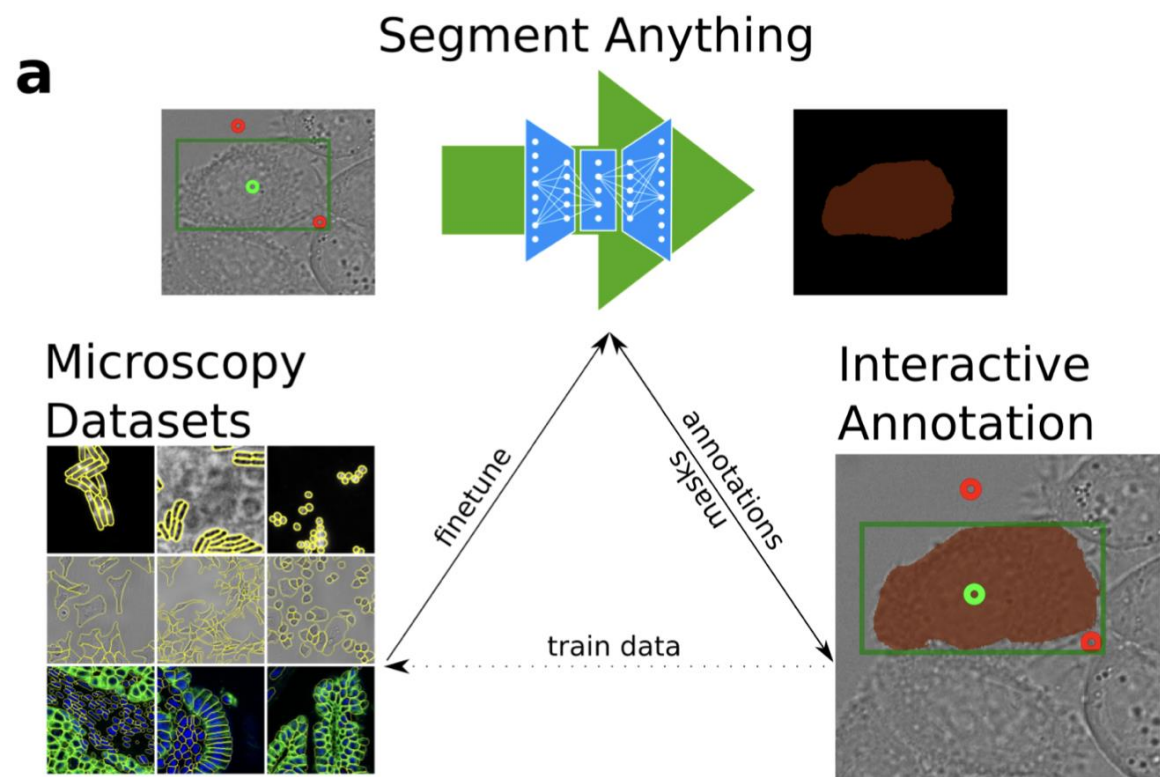
bioRxiv 2023.08.21.554208; doi: <https://doi.org/10.1101/2023.08.21.554208>

Segment Anything Model (SAM) <https://segment-anything.com>

- SAM performs image segmentation tasks by identifying and isolating objects within images automatically, without requiring manual input.
- Interactive & Automatic: Users can either interact with the model by pointing to objects or let it automatically segment everything in an image.
- Trained on a vast dataset, SAM can segment objects in diverse contexts and image types



Segment Anything for Microscopy



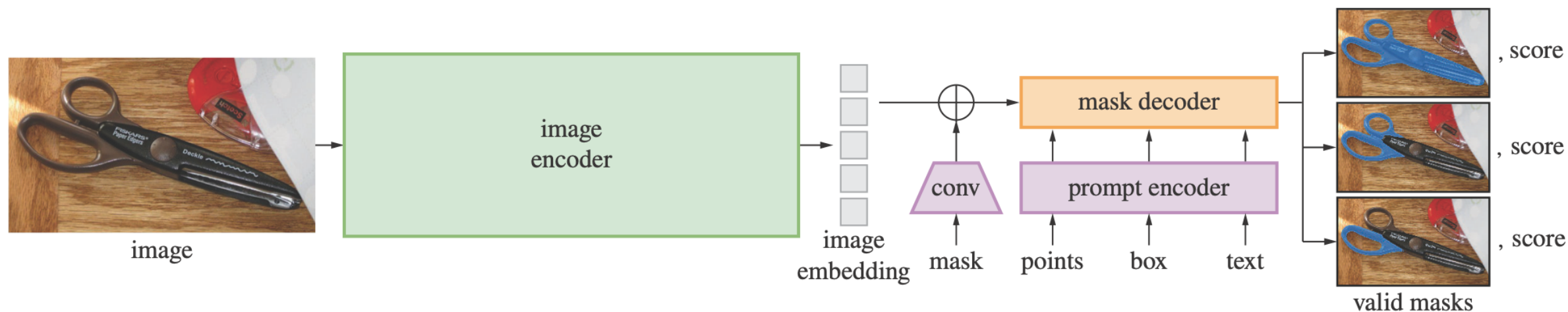
Segment Anything for Microscopy

Anwai Archit, Sushmita Nair, Nabeel Khalid, Paul Hilt, Vikas Rajashekar, Marei Freitag, Sagnik Gupta, Andreas Dengel, Sheraz Ahmed, Constantin Pape

bioRxiv 2023.08.21.554208; doi: <https://doi.org/10.1101/2023.08.21.554208>

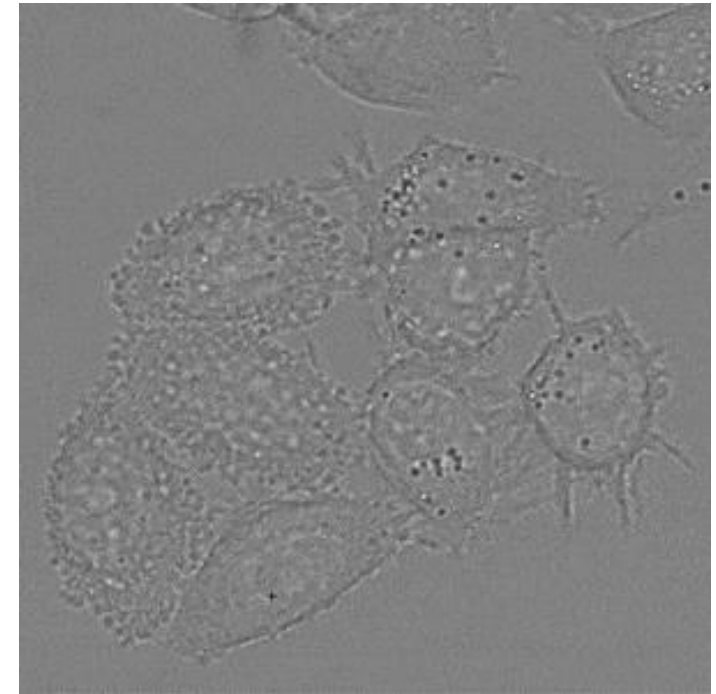
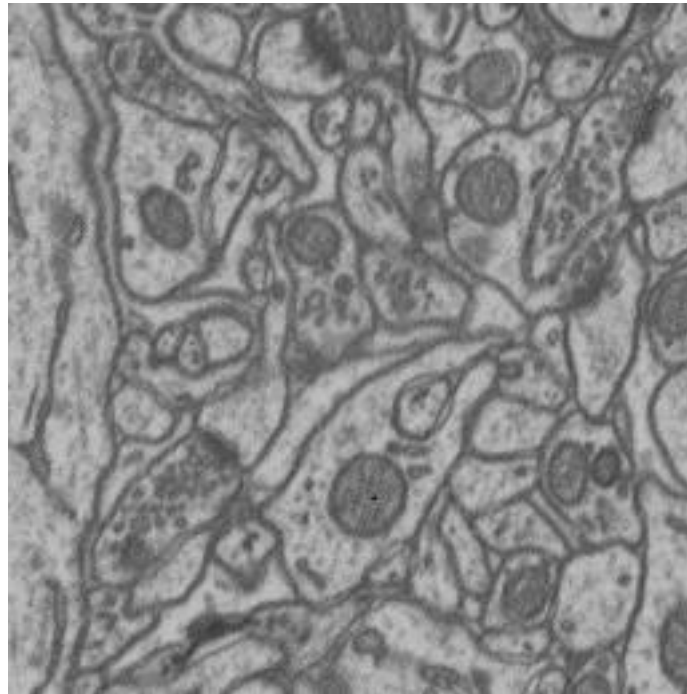
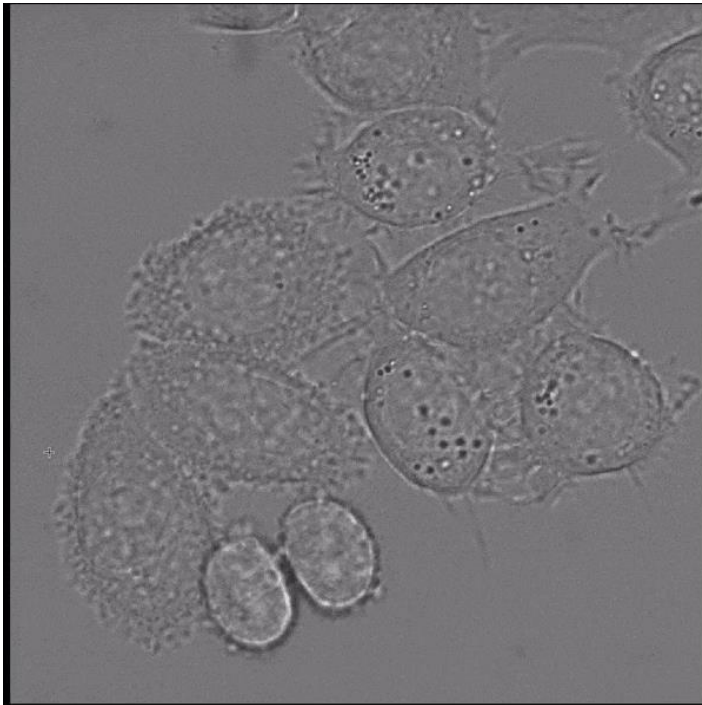
Segment Anything Model (SAM) Overview

- Encoder-Decoder Structure: Utilizes a transformer-based encoder for extracting image features and a decoder that predicts the segmentation mask.
- Promptable Model: Capable of receiving text, points, or bounding boxes as prompts to guide segmentation.
- A ViT image encoder outputs an image embedding that can then be efficiently queried by a variety of input prompts to produce object masks
- For prompts corresponding to more than one object, SAM can output multiple valid masks and associated confidence scores.



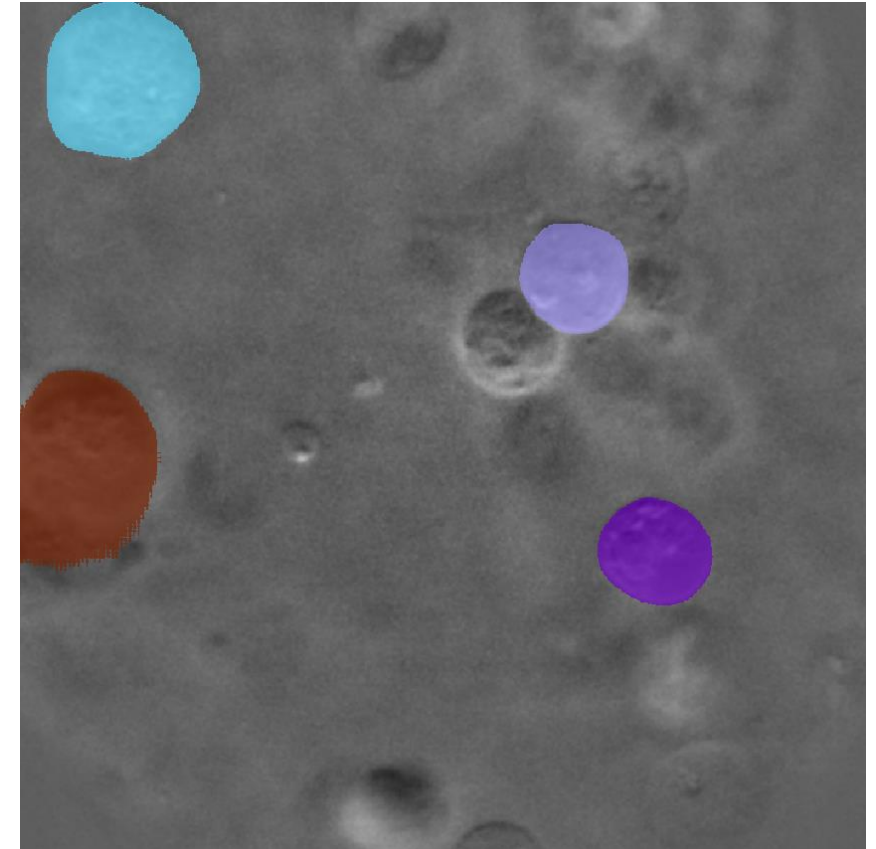
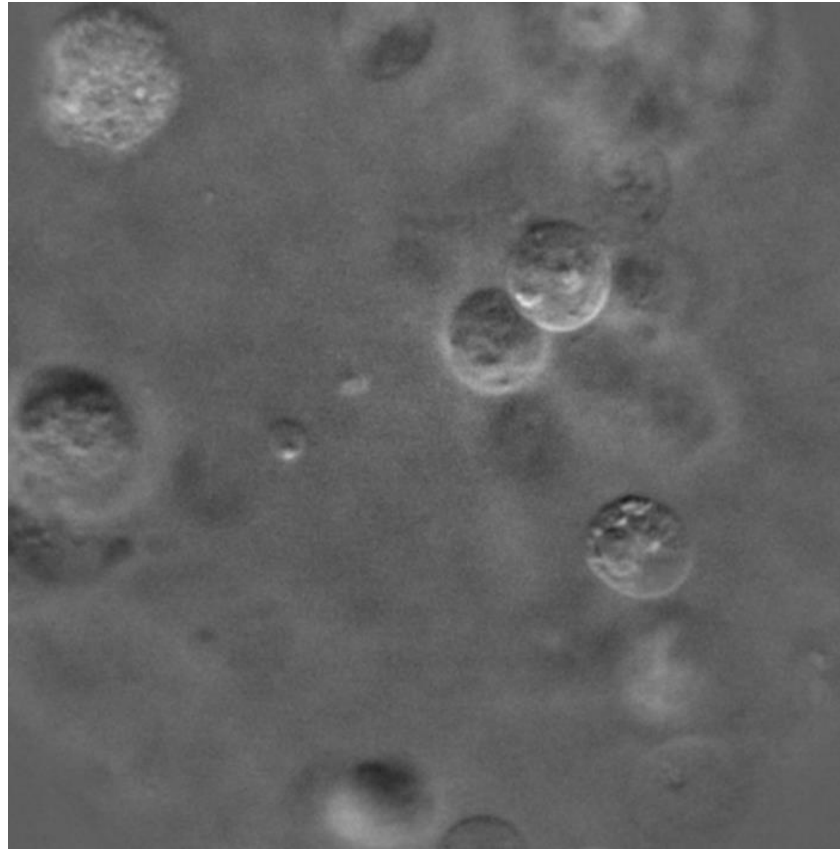
Segment Anything for Microscopy

- <https://github.com/computational-cell-analytics/micro-sam>
- Interactive 2d segmentation (Left: interactive cell segmentation)
- Interactive 3d segmentation (Middle: interactive mitochondria segmentation in EM)
- Interactive tracking of 2d image data (Right: interactive cell tracking)



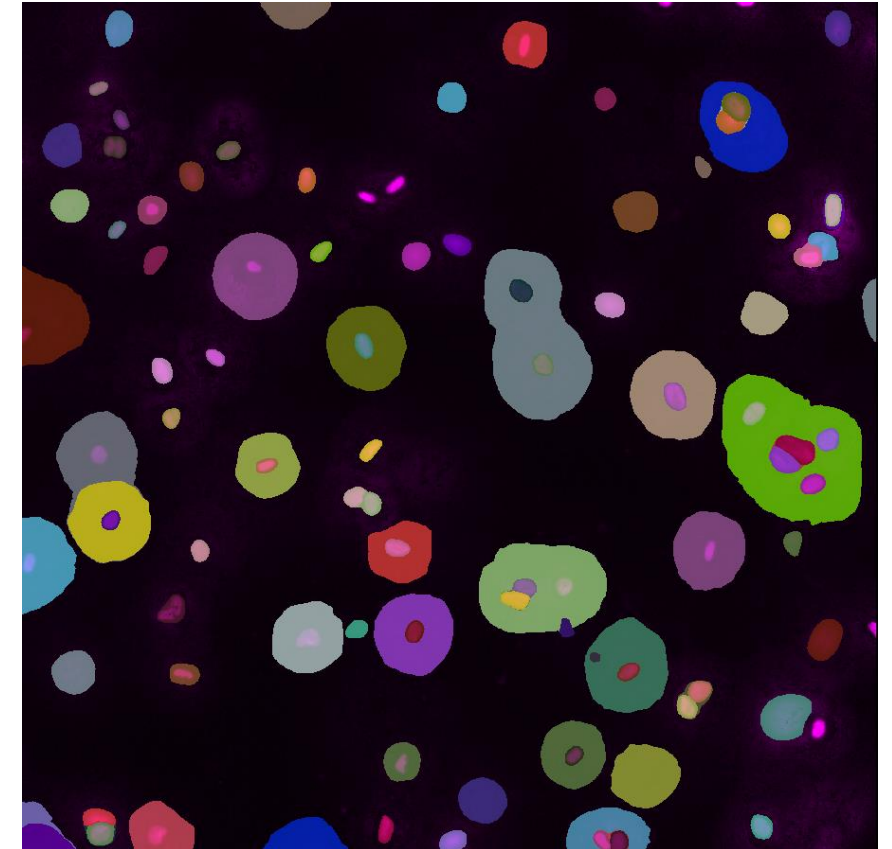
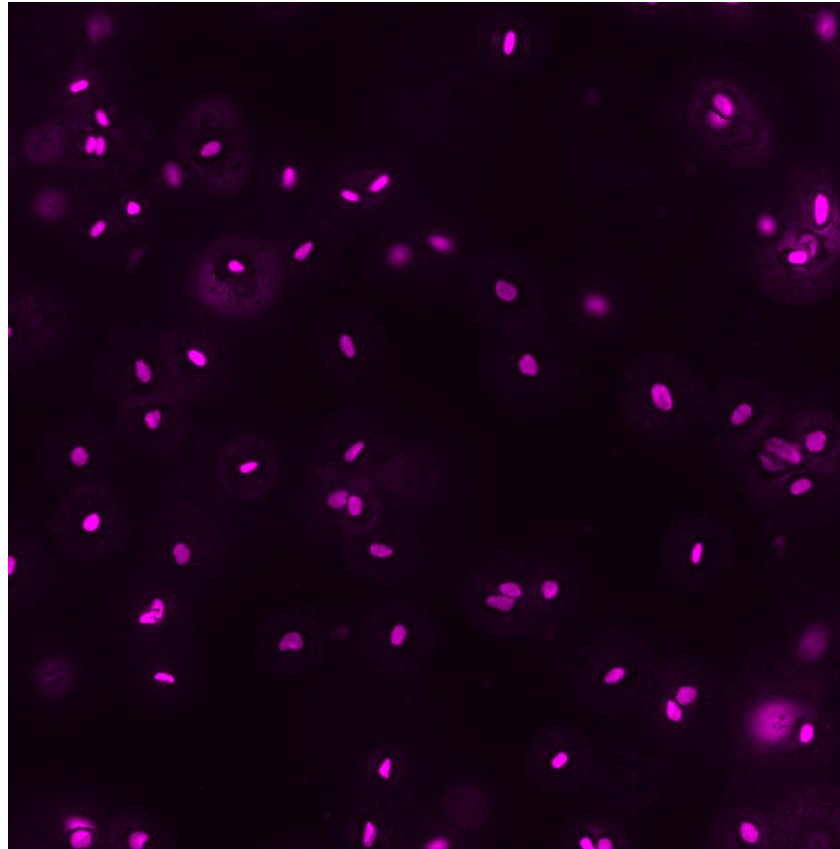
Microsam – CHO cell segmentation

- Runtime on M3 pro: approx. 3s



Microsam – Cell segmentation in 3D culture

- Runtime on M3 pro: approx. 10s



Tools

Python:

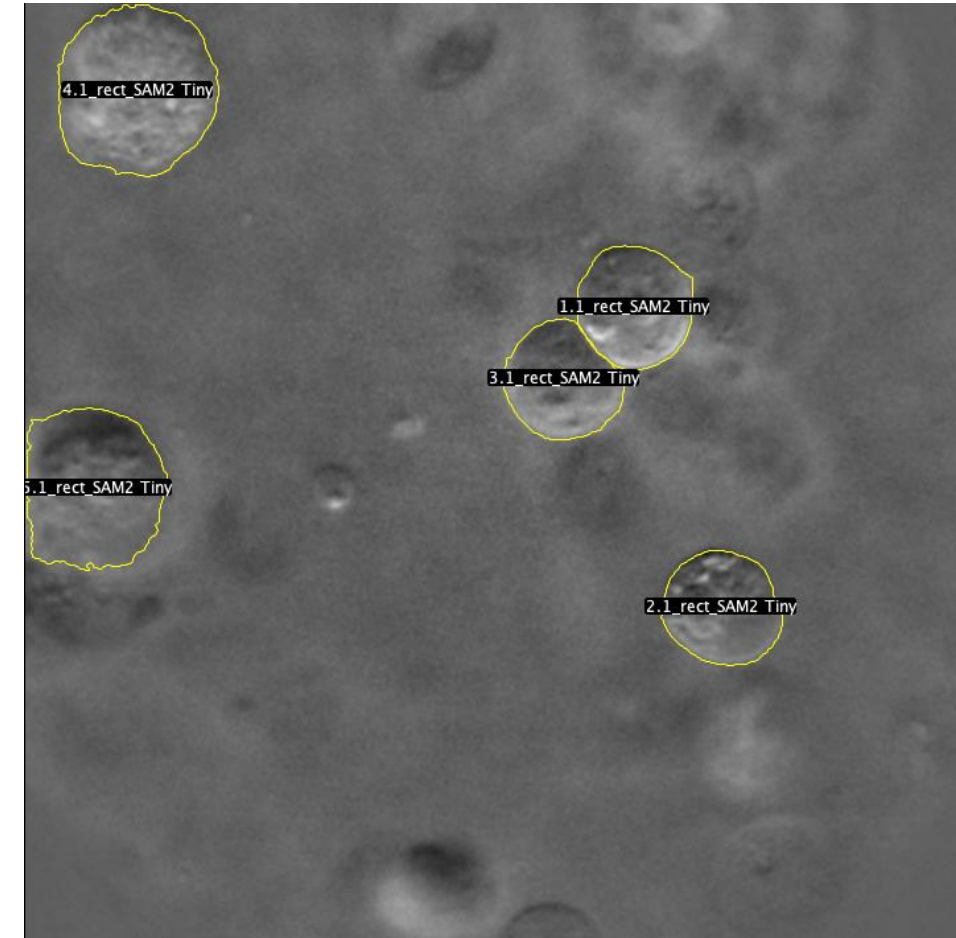
- CV libraries: OpenCV, Scikit-Image, Pillow, Torchvision
- ... or whatever gets the job done

Image Viewer:

- Fiji - distribution of ImageJ, bundling many plugins which facilitate scientific image analysis <https://fiji.sc>
- Napari - a fast, interactive viewer for multi-dimensional images in Python <https://napari.org/>

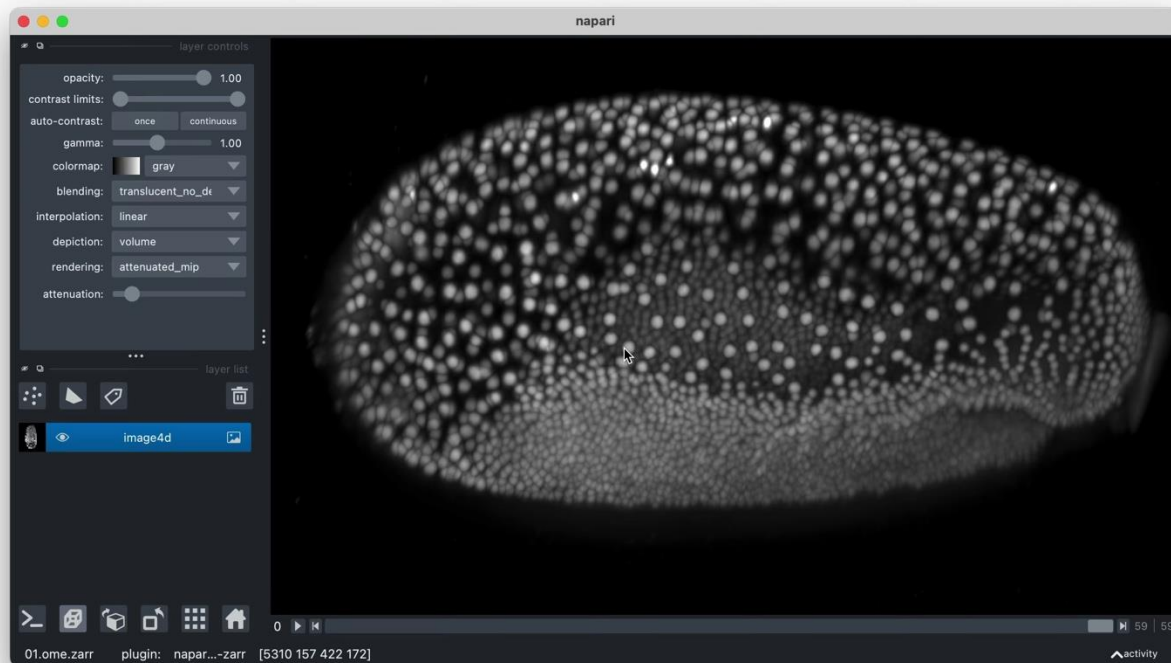
Fiji <https://fiji.sc>

- Bundles together popular plugins for image analysis into one installation
- Automatically manages their dependencies and updating
- Plugins: <https://imagej.net/list-of-extensions>
- Fiji scripting tutorial:
 - <https://syn.mrc-lmb.cam.ac.uk/acardona/fiji-tutorial/>
 - Provide the general idea of how Fiji works
 - How capabilities are organized
 - How can they be composed into a program
- SAMJ-IJ
 - <https://github.com/segment-anything-models-java/SAMJ-IJ>
 - Fiji plugin for annotating microscopy images using various versions of the Segment Anything Model (SAM)



Napari <https://napari.org/stable/index.html>

- Python library for n-dimensional image visualisation, annotation, and analysis
 - View and explore 2D, 3D, and higher-dimensional arrays on a canvas
 - Overlay derived data such as points, polygons, segmentations, and more
 - Annotate and edit derived datasets, using standard data structures such as NumPy or Zarr arrays
- Scripting: https://napari.org/napari-workshop-template/notebooks/intro_bioimage_visualization.html



Napari <https://napari.org/stable/index.html>

- Plugins <https://napari.org/napari-workshop-template/plugins.html>
 - Python packages that allow developers to extend napari functionality
 - Repository for searching/discovering plugins <https://www.napari-hub.org>
 - Plugins can be installed as Python packages using conda or pip as normal or from within the napari GUI using the Plugin menu
-
- Napari – Microsam <https://computational-cell-analytics.github.io/>
 - Napari – MEDIAR <https://github.com/joonkeekim/mediar-napari>