A comparison of super-resolution microscopy approaches to undertake deep learning-based 3D shape analysis of *Orientia tsutsugamushi* (Ot) bacteria inside mammalian cells

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A comparison of super-resolution microscopy approaches to undertake deep learning-based 3D shape analysis of *Orientia tsutsugamushi* bacteria inside mammalian cells

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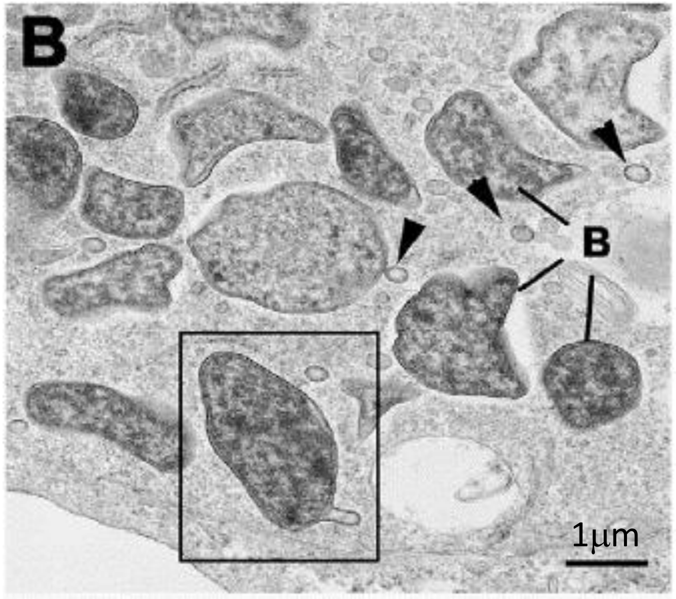
Manuscript in preparation!

Orientia tsutsugamushi (Ot) bacteria

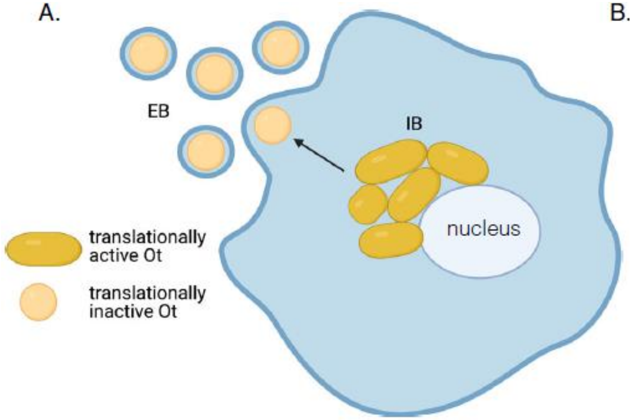
- Mite-borne gram-negative bacterium responsible for a disease called scrub typhus in humans
- Small bacteria present as dense, intracellular clumps in infected cells



Ot bacteria in U-937, human monocyte cell line

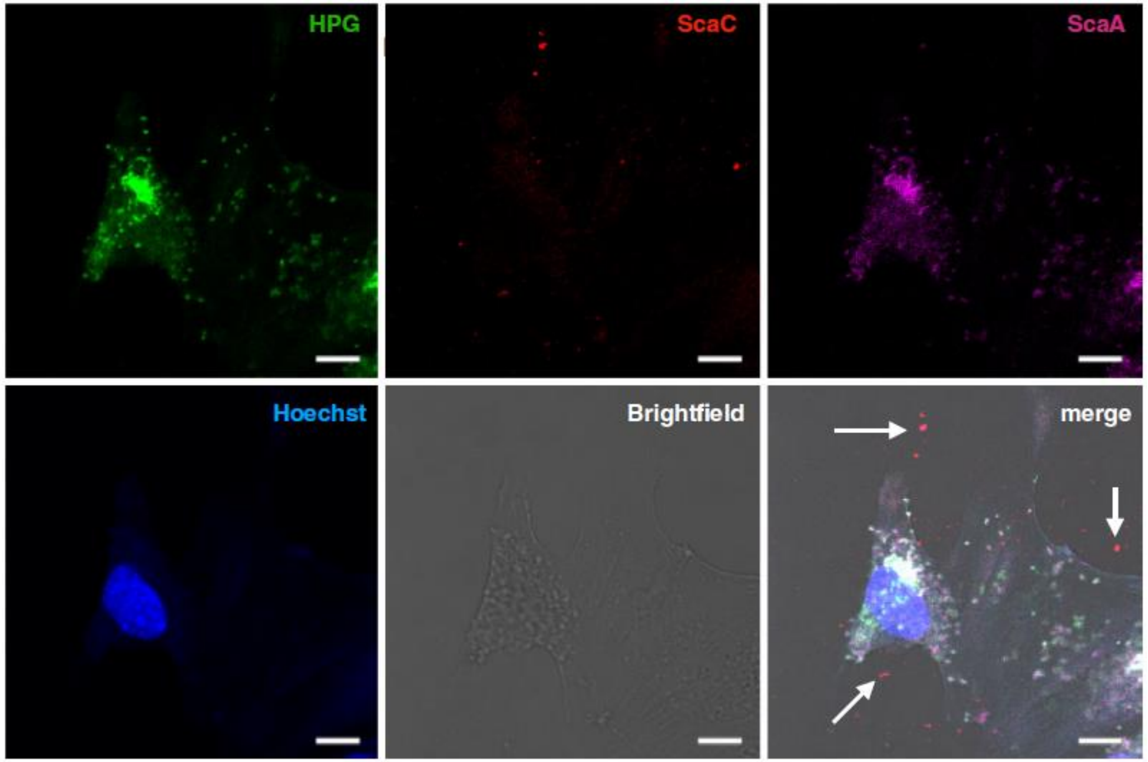
Paris et al. 2012 PMID: 22253938

Biological question



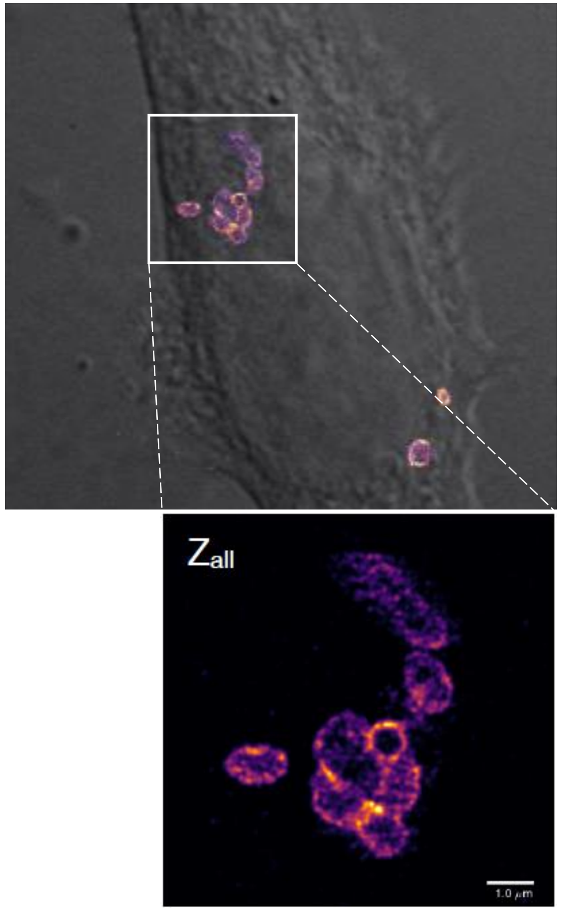
Intracellular bacteria (IB)
vs
Extracellular bacteria (EB)

Atwal et al. 2022 PMID: 35739103


Translation marker

Bacterial surface proteins

Confocal microscopy


DIC + STORM

STORM

- Confocal microscopy proved insufficient to resolve individual intracellular bacteria (for counting or shape analysis)
- STORM microscopy showed clear boundary for only a subset of cells
- Salje lab collaborated with us to test which of our facility super-resolution microscopes would be best to image the bacteria and quantify size (volume) and shape

Microscopes used in this study

Modality	Confocal	iSIM	Airyscan	3D-SIM	STED
System	Zeiss LSM 880	VisiTech InstantSIM	Zeiss LSM 880 with Airyscan detector	Structured Illumination Microscope from Applied Precision/OMX	Stimulated Emission Depletion microscope from Abberior
Objective	63x/1.4 NA	100x/1.4 NA	63x/1.4 NA	100x/1.4 NA	100x/1.4 NA 100x/1.45 NA
Image processing during acquisition	None	Deconvolution using Micovolution module	Wiener deconvolution	Wiener deconvolution	None

All post-processing was part of the acquisition software

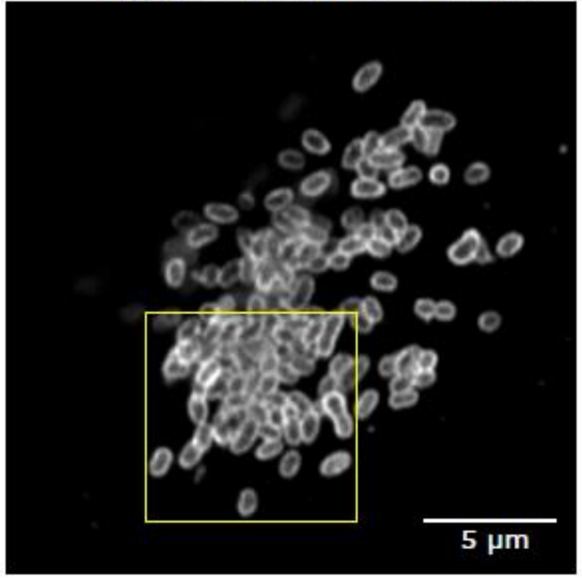
Images acquired on different microscopes

HUVEC and HeLa cells infected with the Ot bacteria

Fixed bacteria stained with the membrane marker ScaA

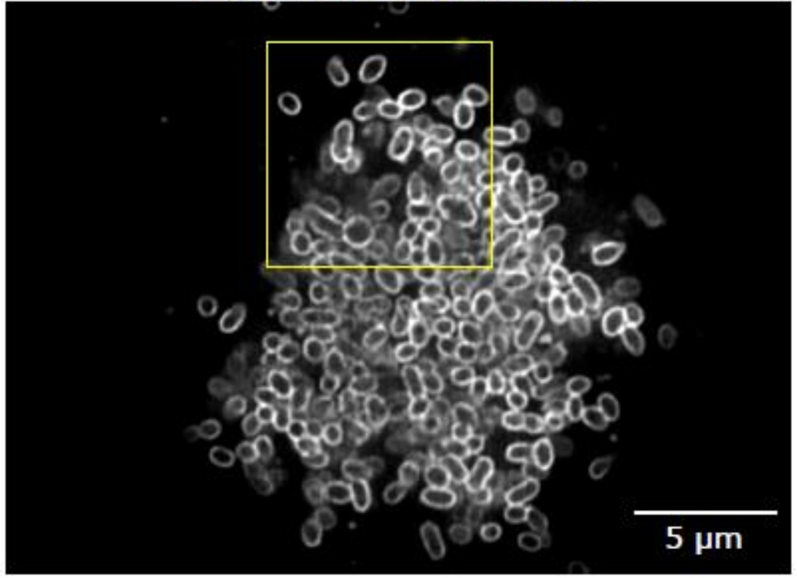
Confocal

Original size:1024 x 1024 px



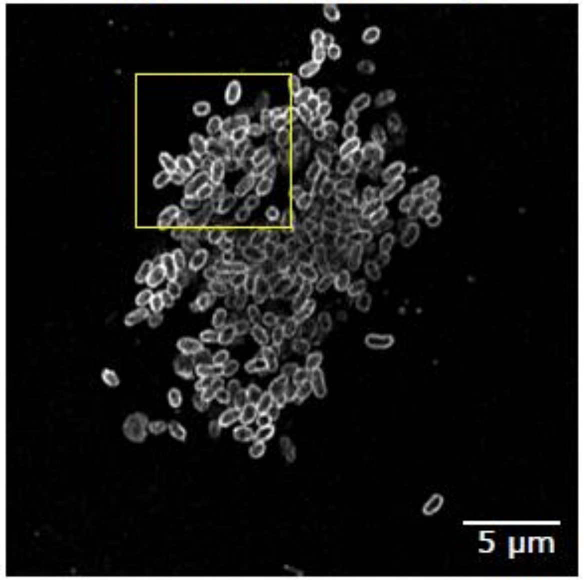
iSIM

Original size: 860 x 624 px



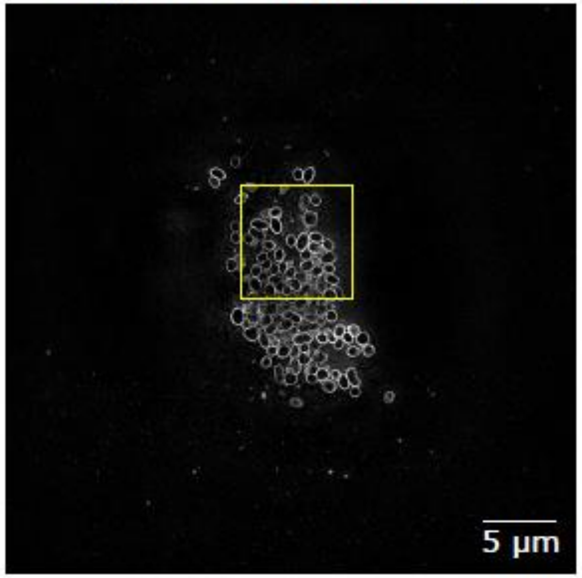
Airyscan

Original size:744 x 744 px



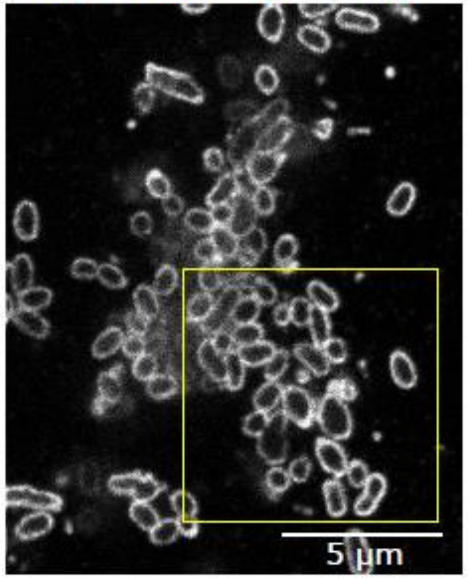
3D-SIM

Original size:1024 x 1024 px

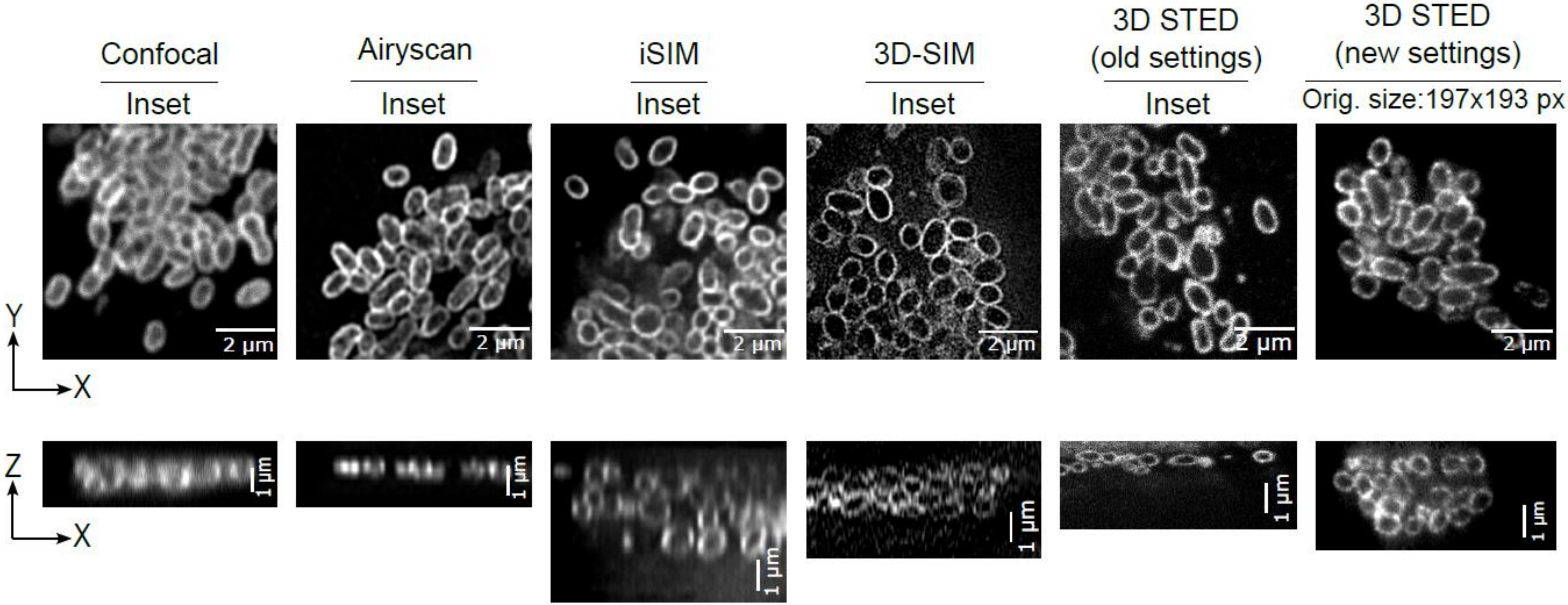


3D STED (old settings)

Original size: 575 x 719 px



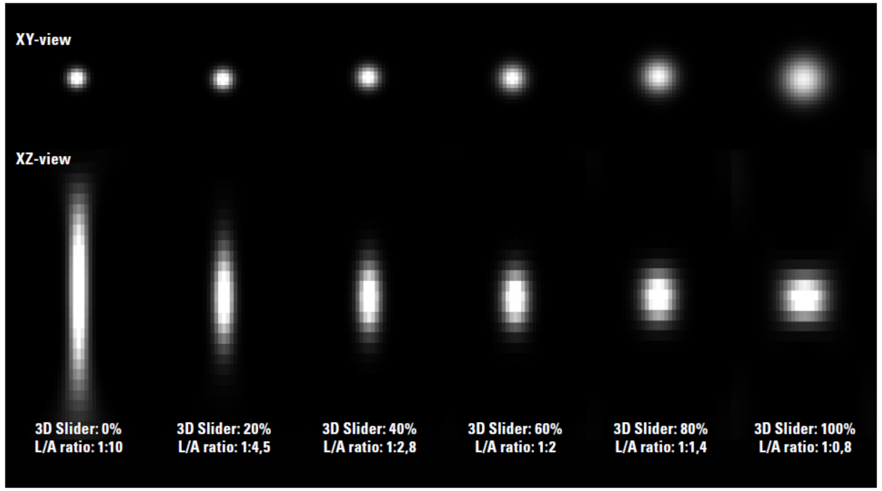
Comparison of microscopy modalities for imaging Ot bacteria

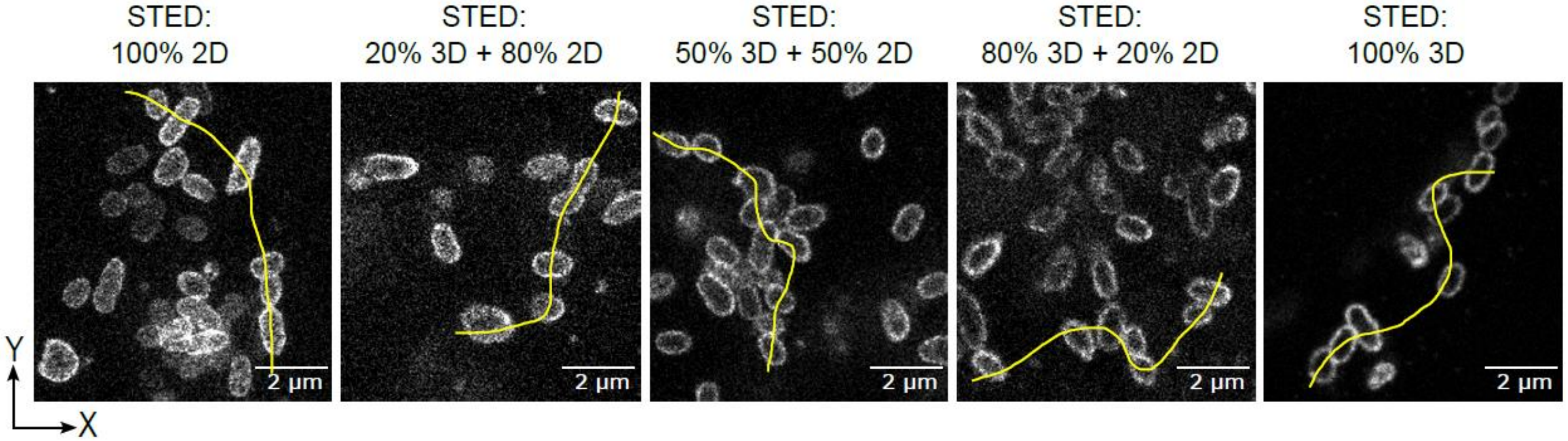


3D-STED gave the best isotropic resolution.

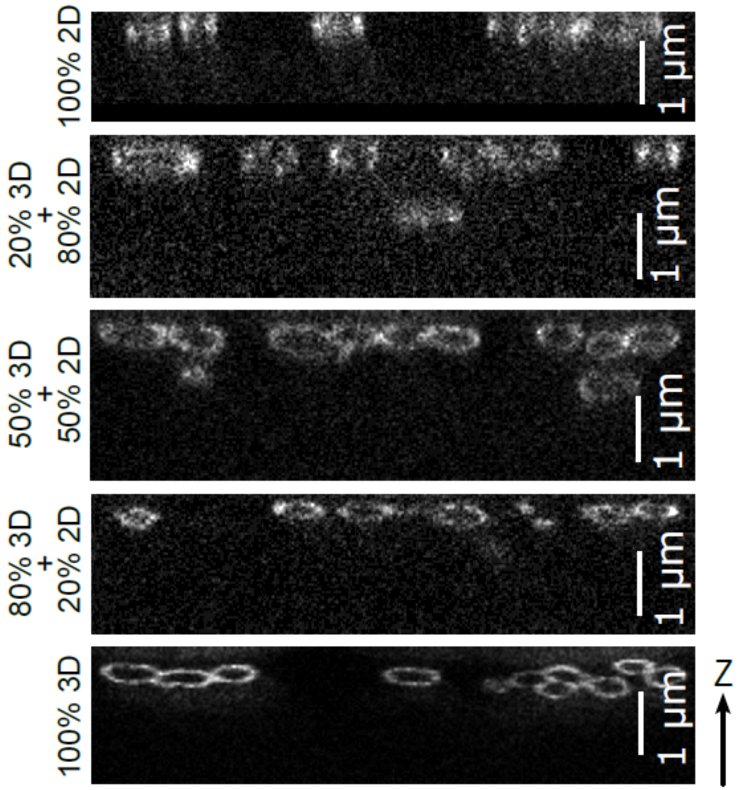
3D-STED for isotropic resolution

PSFs with increasing values of 3D STED values





Orthogonal view along the yellow lines



- 2D-STED images appeared to show a diffuse label all over the bacteria, rather than a clear localization at the periphery of the bacteria;
- combined different proportions of 2D-STED and 3D-STED

Evaluation of existing Deep Learning 3D segmentation methods

ARTICLE

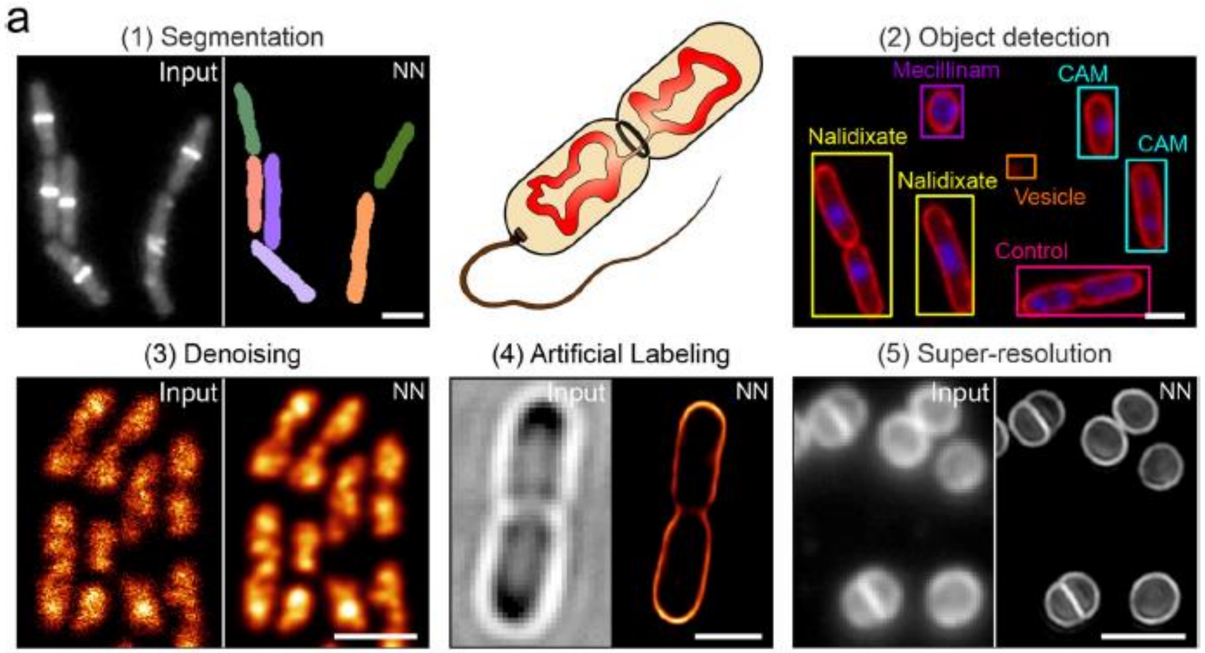
Check for updates

https://doi.org/10.1038/s42003-022-03634-z

OPEN

DeepBacs for multi-task bacterial image analysis using open-source deep learning approaches

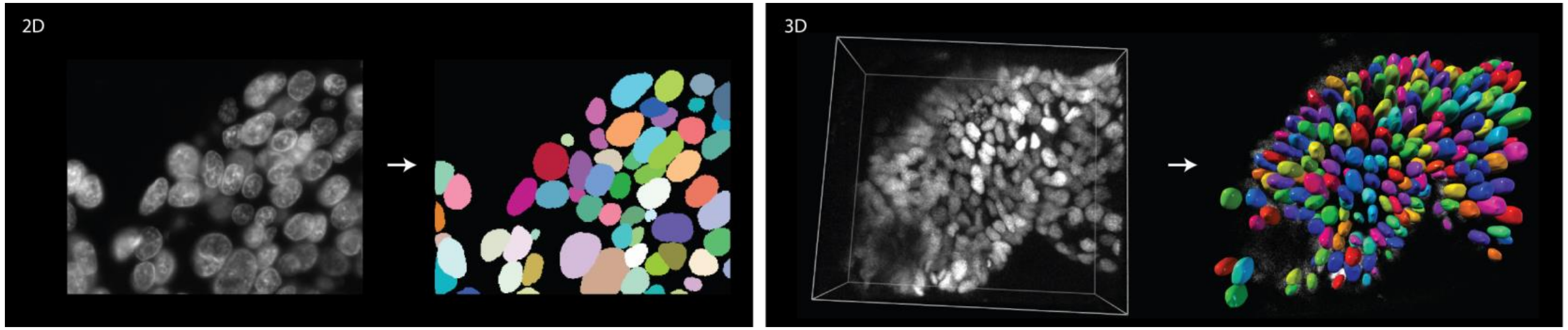
Christoph Spahn[1,2]✉, Estibaliz Gómez-de-Mariscal[3], Romain F. Laine[4,5,11], Pedro M. Pereira[6], Lucas von Chamier[4], Mia Conduit[7], Mariana G. Pinho[6], Guillaume Jacquemet[8,9,10], Séamus Holden[7], Mike Heilemann[2]✉ & Ricardo Henriques[3,4,5]✉



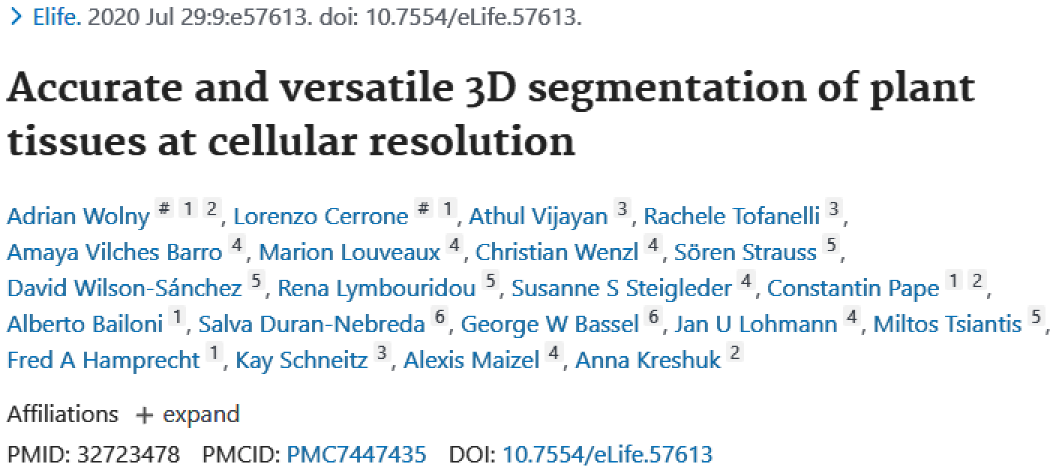
DeepBacs does not do 3D segmentation!

StarDist and PlantSeg

StarDist – star-convex object detection in 2D and 3D images



StarDist did not work with our bacteria membrane staining images



> Elife. 2020 Jul 29:9:e57613. doi: 10.7554/eLife.57613.

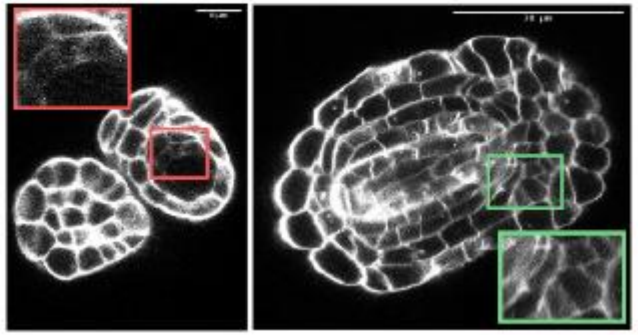
Accurate and versatile 3D segmentation of plant tissues at cellular resolution

Adrian Wolny[#,1,2], Lorenzo Cerrone[#,1], Athul Vijayan[3], Rachele Tofanelli[3], Amaya Vilches Barro[4], Marion Louveaux[4], Christian Wenzl[4], Sören Strauss[5], David Wilson-Sánchez[5], Rena Lymbouridou[5], Susanne S Steigleder[4], Constantin Pape[1,2], Alberto Bailoni[1], Salva Duran-Nebreda[6], George W Bassel[6], Jan U Lohmann[4], Miltos Tsiantis[5], Fred A Hamprecht[1], Kay Schneitz[3], Alexis Maizel[4], Anna Kreshuk[2]

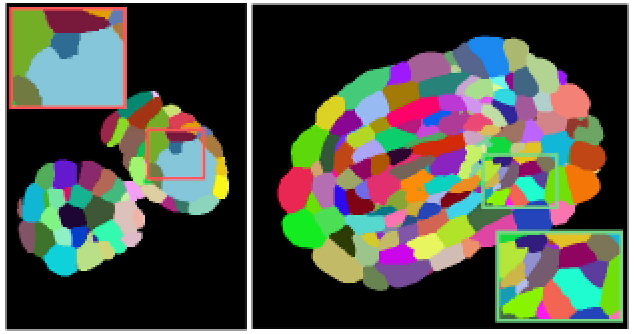
Affiliations + expand

PMID: 32723478 PMCID: PMC7447435 DOI: 10.7554/eLife.57613

Input image



3D segmentation with PlantSeg



PlantSeg did not work with our bacteria images.

Cellpose

› Nat Methods. 2021 Jan;18(1):100-106. doi: 10.1038/s41592-020-01018-x. Epub 2020 Dec 14.

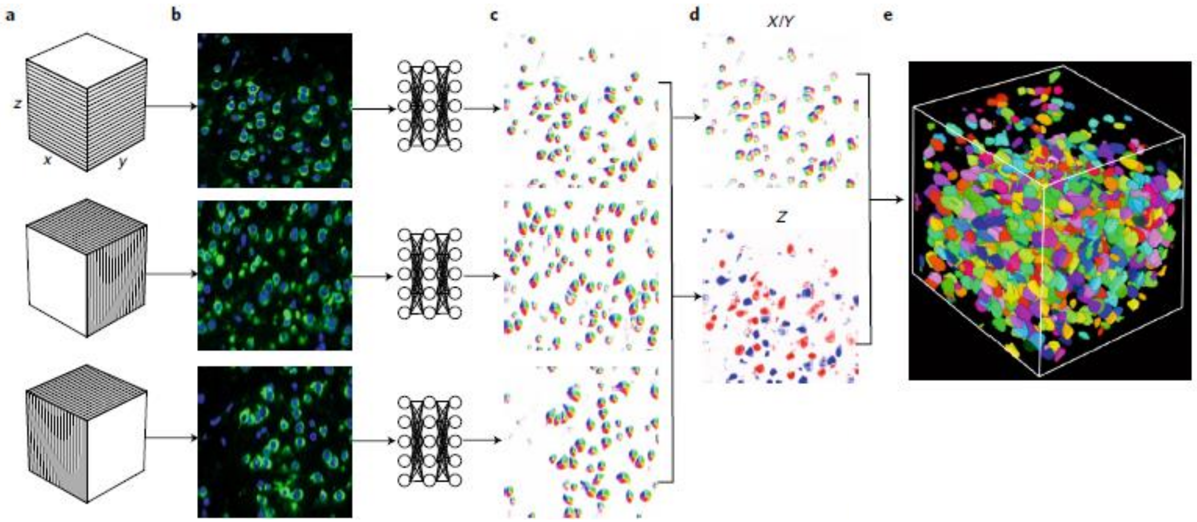
Cellpose: a generalist algorithm for cellular segmentation

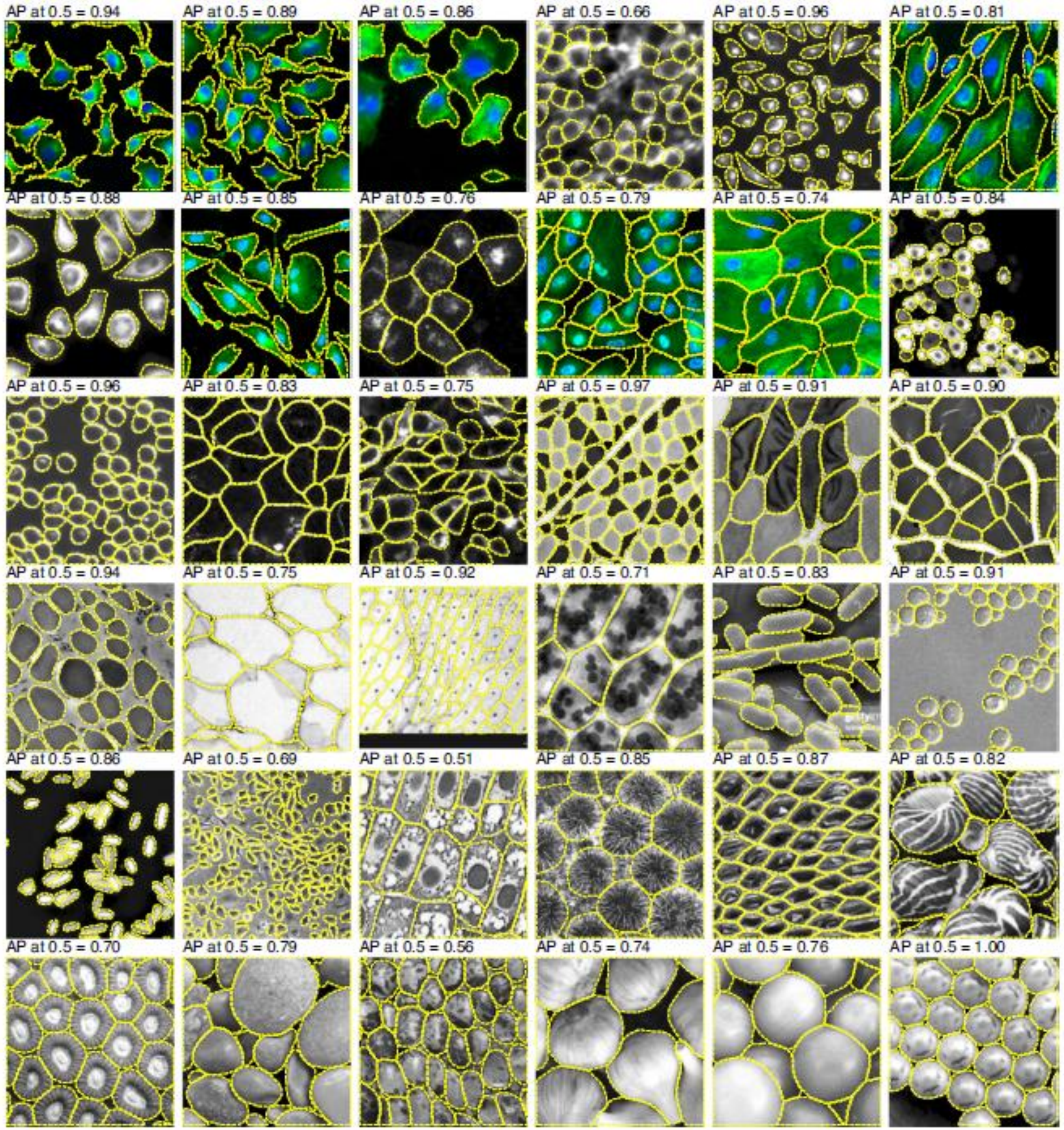
Carsen Stringer [1], Tim Wang [1], Michalis Michaelos [1], Marius Pachitariu [2]

Affiliations + expand

PMID: 33318659 DOI: 10.1038/s41592-020-01018-x

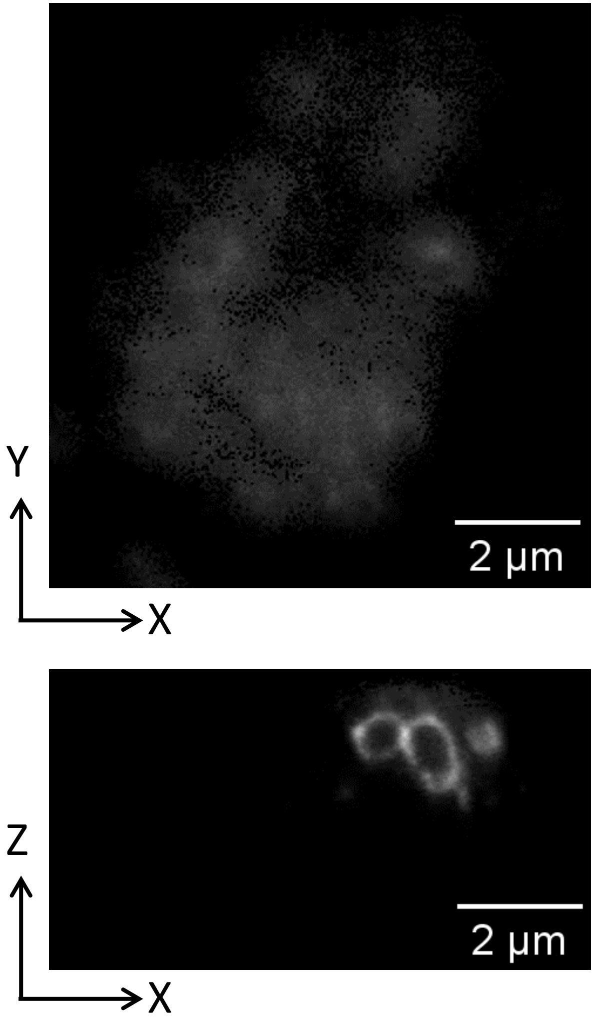
3D segmentation



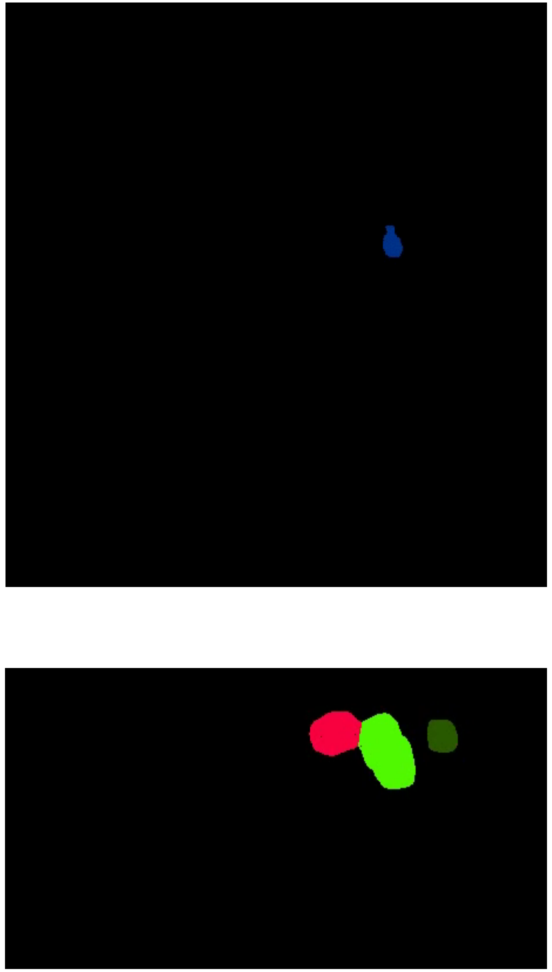


3D segmentation with Cellpose

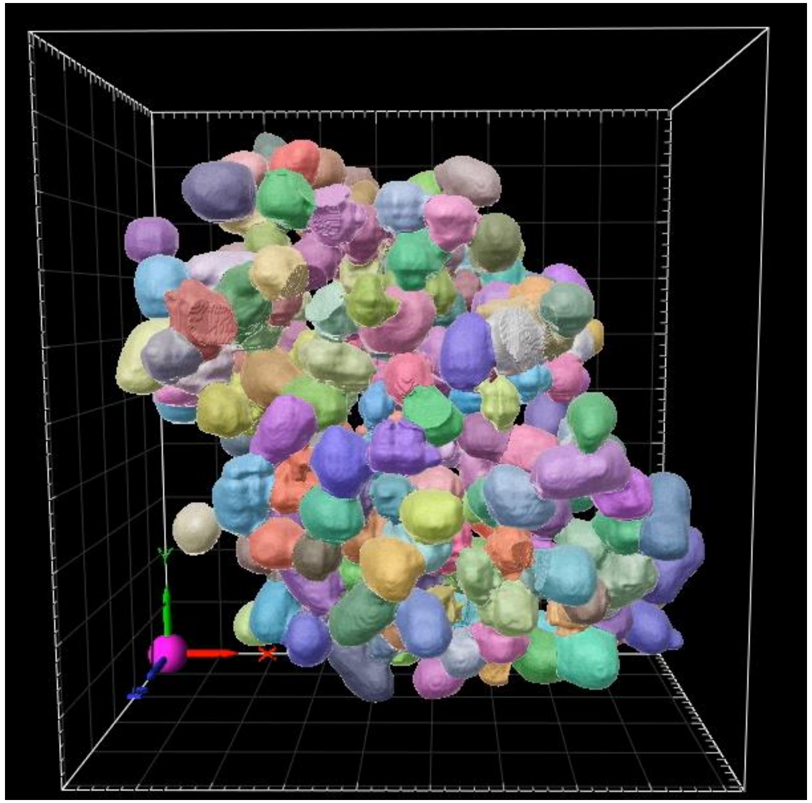
3D STED images



Cellpose Segmentation

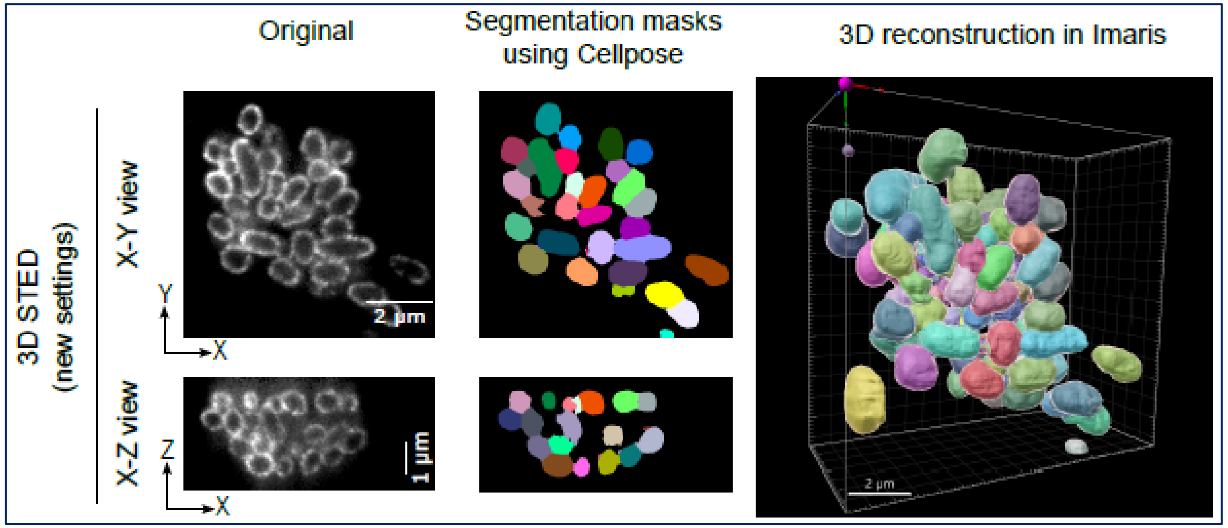


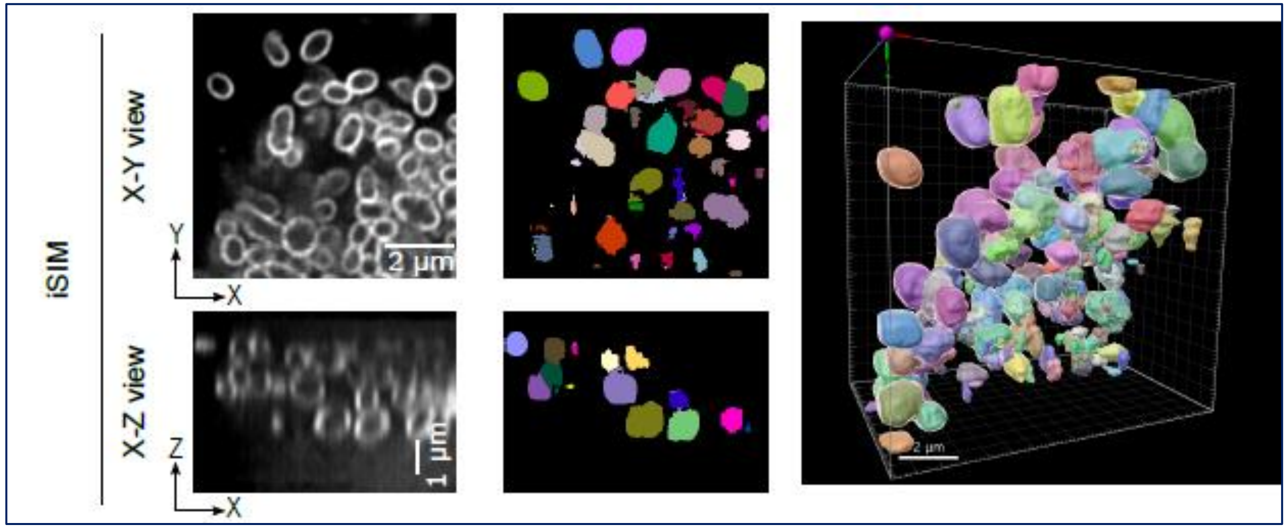
Imaris 3D reconstruction

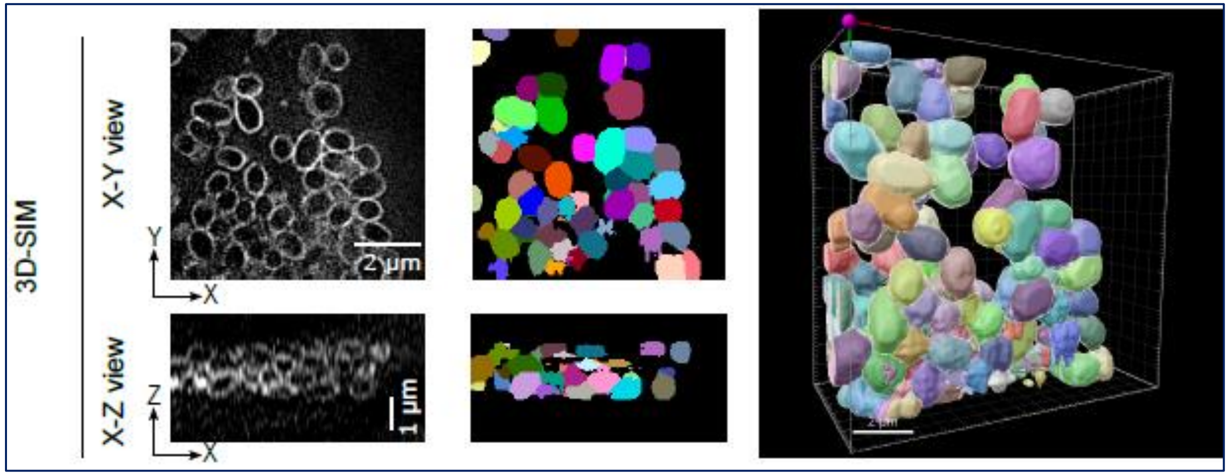


Cellpose gave the best results for our bacterial images!

3D segmentation results for 3D-STED vs iSIM vs 3D-SIM images

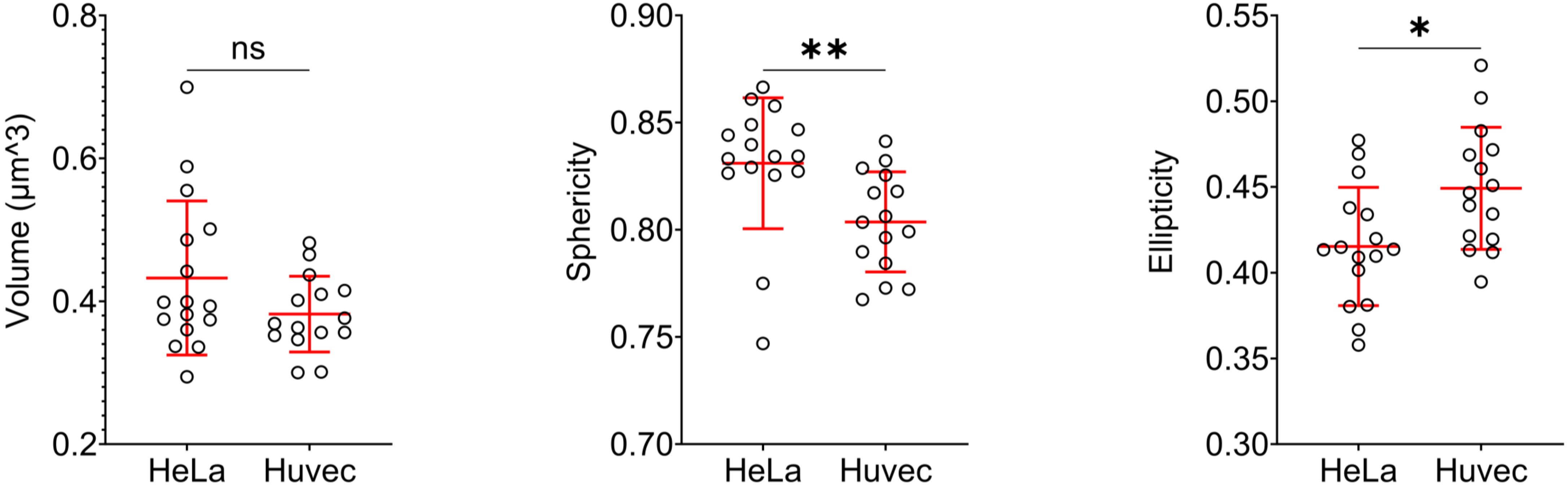






- 3D-STED images gave the best bacterial 3D segmentation
- This allowed us to count and analyze the size/shape of bacteria.
- iSIM and 3D-SIM images gave sub-optimal 3D segmentation results

Size and shape quantification of Ot bacteria



Summary

- Imaging Ot bacteria is challenging due to its small size (1-2 μm in diameter)
- We tested different super-resolution microscopes (Airyscan, iSIM, 3D-SIM, STED) and compared it with images from a regular confocal microscope

Caveats

- For 3D segmentation, used images acquired from the microscopes; did not do any noise reduction (deconvolution, denoising)
- Did not try classical or machine learning 3D segmentation
- Did not train a new machine/deep model for our data

- Tried existing deep learning methods and found Cellpose to be optimal for 3D segmentation of our bacteria images
- We were able to quantify bacteria size and shape parameters

Acknowledgement

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Graham Wright
John Lim

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