

Supplementary Figures for Benchmarking Cell Segmentation in Two Spatialomics Platforms with Open-source Software

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and Andrew C. Nelson^{*}

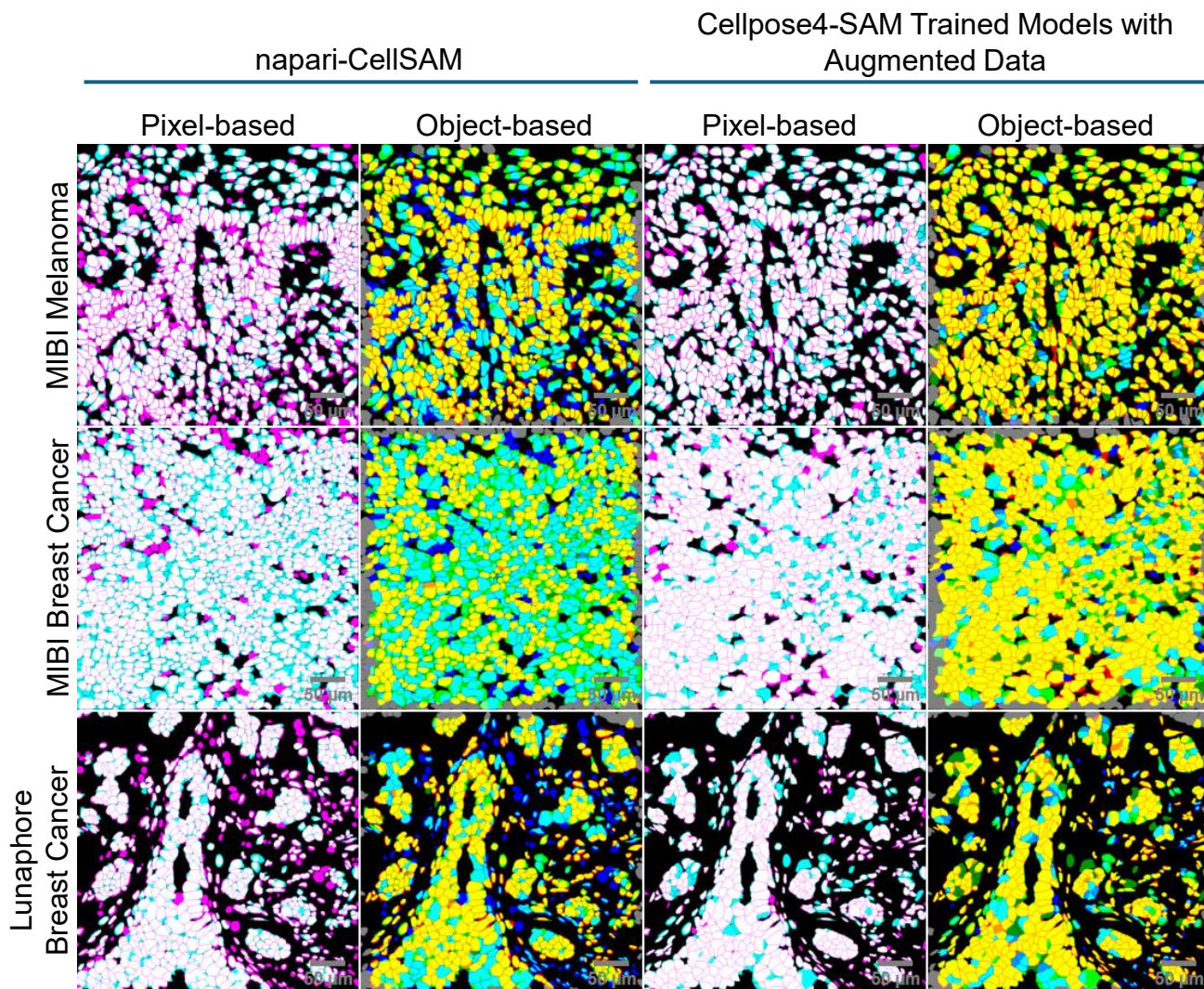
[#]Equal contribution as co-first authors.

^{*} Corresponding author.

Supplementary Table S1. Colors indicate the different types of object identification in the object-based analyses by the FIJI Mask Instant Comparator plugin.

Color Indicator	Description
TP	Overlap between GT and test objects
TP	Test object is smaller than GT object
TP	Test object is larger than GT object
FN	GT object without a matching test object
FP	Test object without a matching GT object
FN	Merging of multiple GT objects into a single test object, one of whose GT objects is validated by the given IoU threshold. One of the objects in the GT mask will be yellow and the others orange.
FP	Merging of multiple GT objects into a single test object, one of whose GT objects has a match, but is not validated by the given IoU threshold. One of the GT objects will be cyan, while the others are royal blue.
FP and FN or FP	- GT and test objects overlap, but are not validated by the given IoU threshold. - FPs corresponding to the division of one GT object into several test objects. One of the test objects will be yellow or cyan (depending on validation at the given IoU threshold), while the others will be cyan.
	Object not evaluated due to proximity of its centroid to the image border.

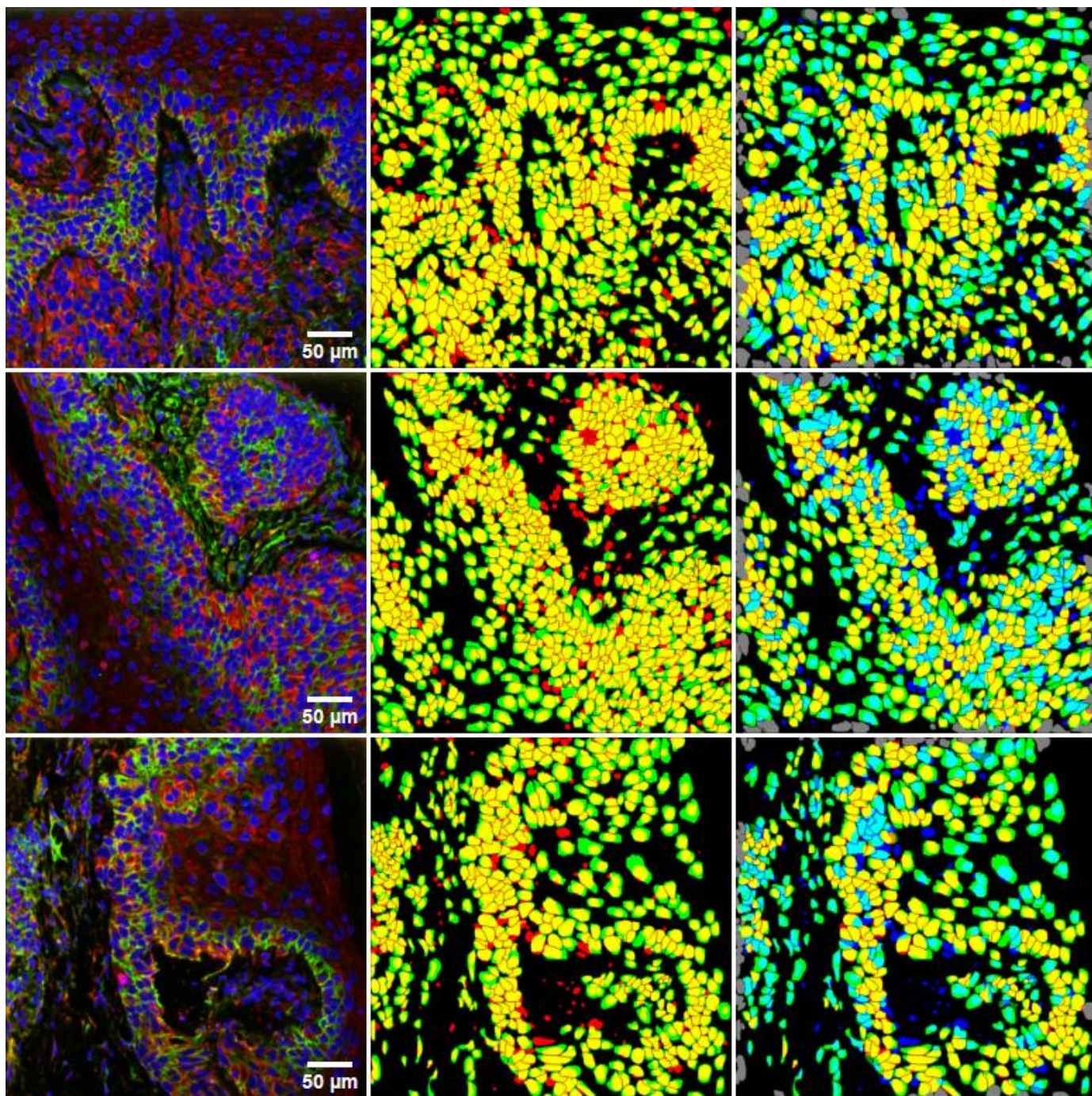
Supplementary Figure S1. Color-blind Example of Figure 2 in Main Text.



Suppl. Figure 2. Representative images showing pixel- and object-based segmentation images. This figure shows one representative image from each dataset segmented by the napari-CellSAM model (left) and the Cellpose4-SAM models trained with augmented data (right). For pixel-based segmentations (the first and third columns), white represents TP pixels, cyan represents FN pixels, and magenta represents FP pixels. For object-based segmentations (the second and fourth columns), yellow represents overlap between GT and predicted objects; bright green signifies that the predicted object is smaller than the GT object; red indicates the predicted object is larger than the GT object; dark green indicates GT object without a matching predicted object; dark blue represents a predicted object without a matching GT object; cyan indicates that the GT and predicted objects overlap, but are not validated by the given IoU threshold (0.5 for above images), or the division of one GT object into several predicted objects; for the remaining colors, please see **Supplementary Table S9**.

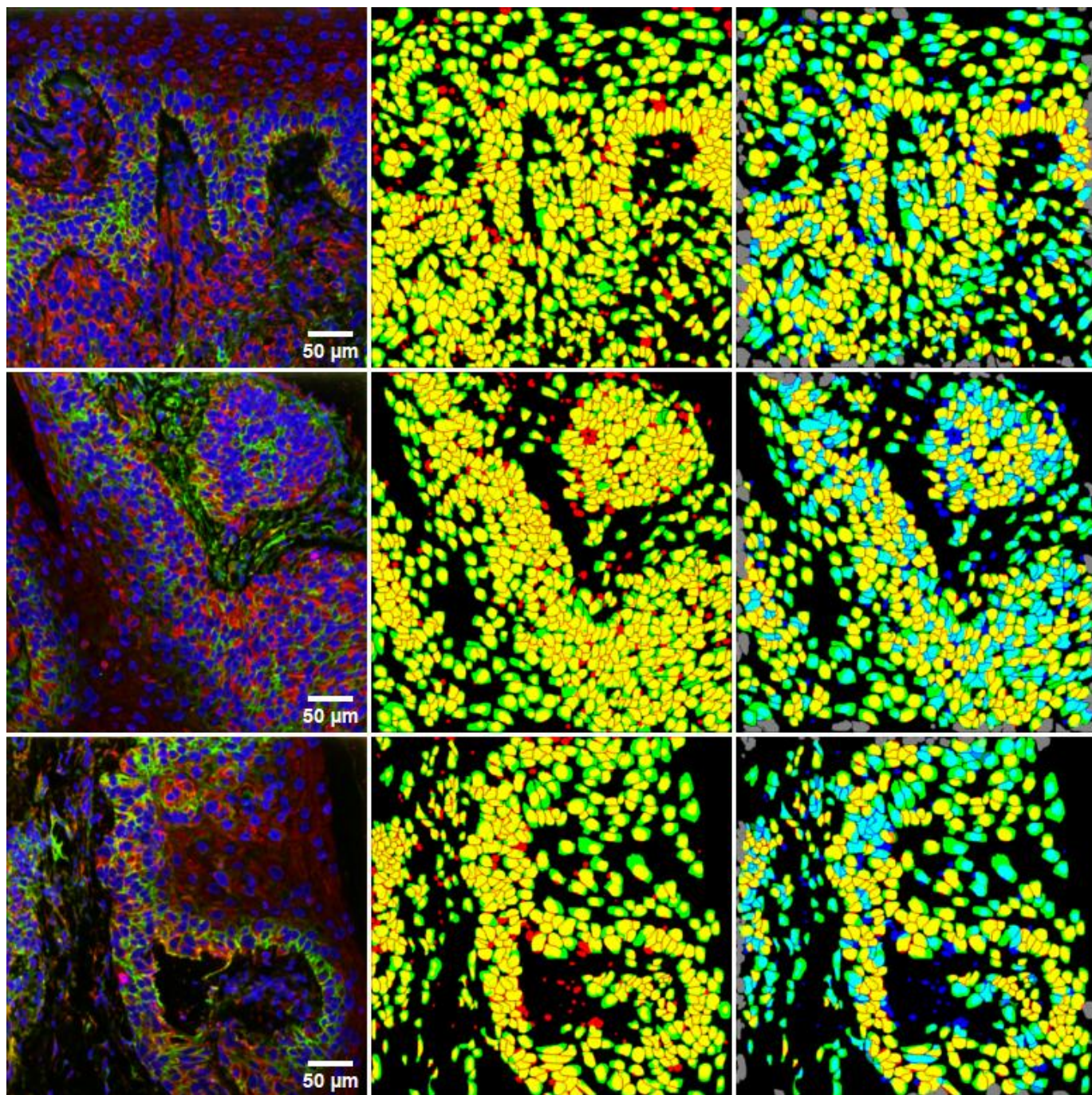
Supplementary Figures for Melanoma MIBI Images

Supplementary Figure S2. Denoised, Contrast-enhanced Melanoma MIBI Images with Pixel- and Object-based Segmentations by the Cellpose3-Cyto3 Model



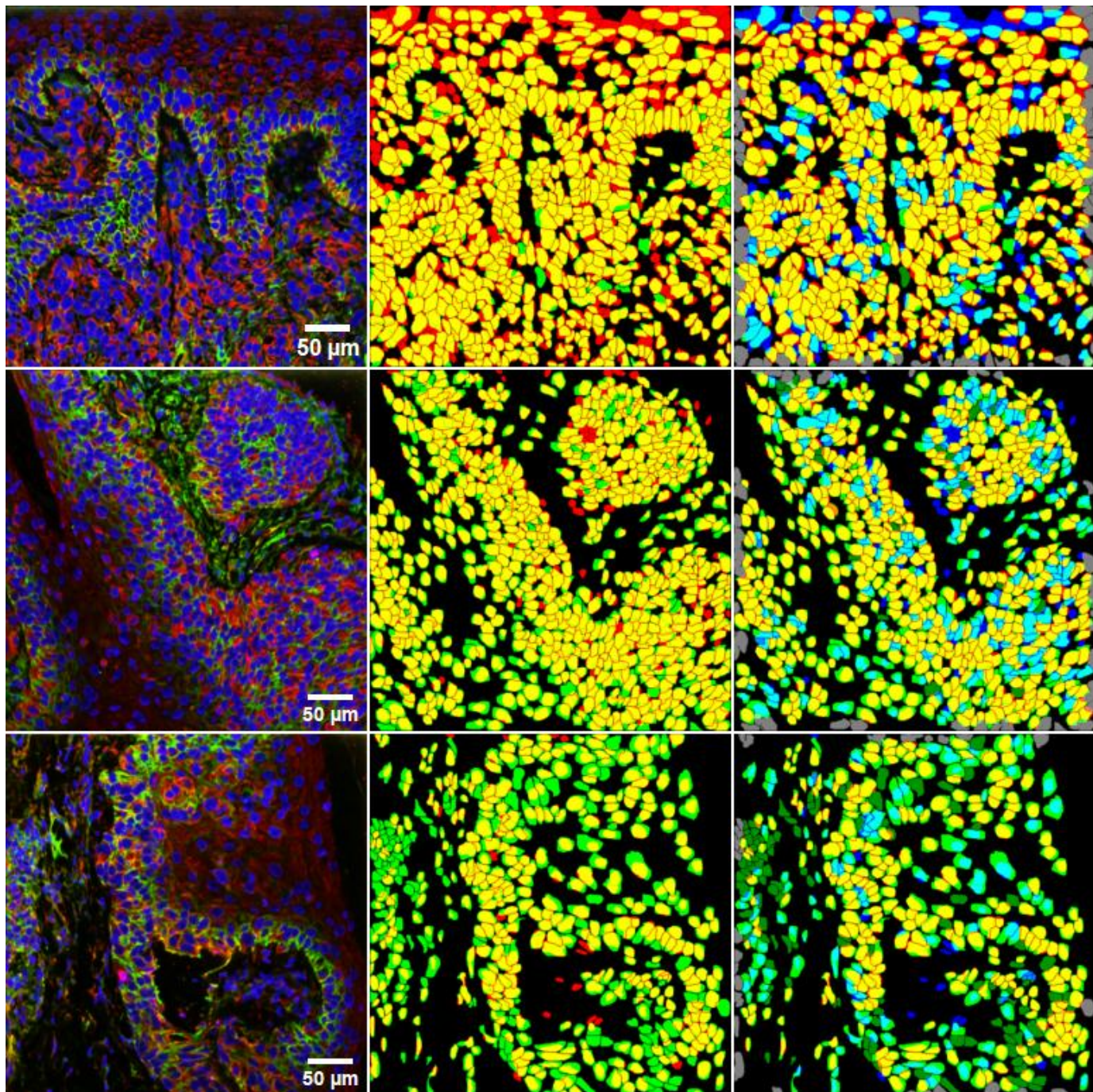
Top row: *Left:* Denoised, contrast-enhanced melanoma MIBI Image 1. *Middle:* Pixel-based segmentation analysis (IoU=0.5) by Cellpose3-Cyto3 on melanoma MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose3-Cyto3 on melanoma MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 2. *Middle:* Pixel-based segmentation analysis by Cellpose3-Cyto3 on melanoma MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose3-Cyto3 on melanoma MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 3. *Middle:* Pixel-based segmentation analysis by Cellpose3-Cyto3 on melanoma MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose3-Cyto3 applied to melanoma MIBI Image 3.

Supplementary Figure S3. Denoised, Contrast-enhanced Melanoma MIBI Images with Pixel- and Object-based Segmentations by the Cellpose3-TissueNet Model



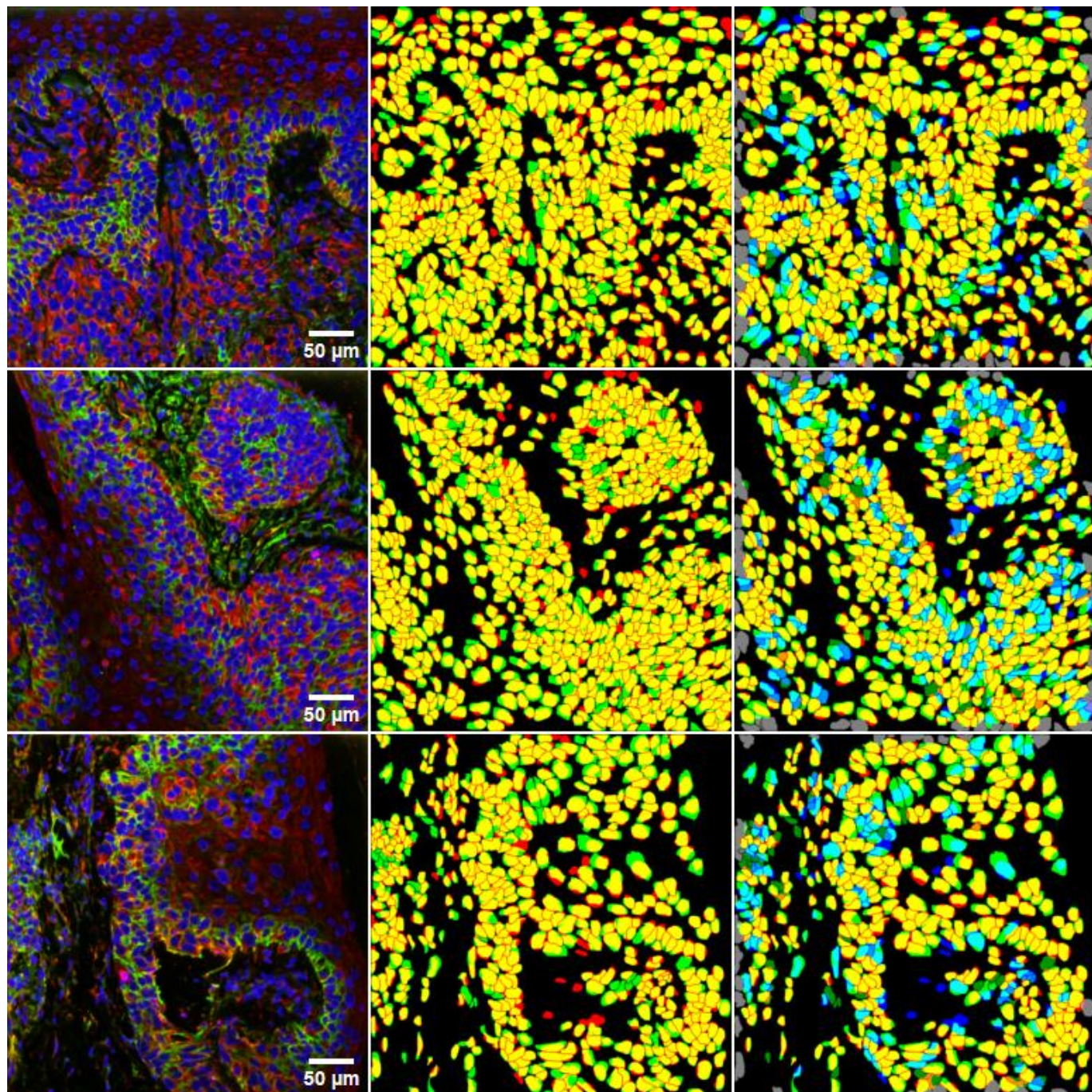
Top row: *Left:* Denoised, contrast-enhanced melanoma MIBI Image 1. *Middle:* Pixel-based segmentation analysis by Cellpose3-TissueNet on the melanoma MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose3-TissueNet on melanoma MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 2. *Middle:* Pixel-based segmentation analysis by Cellpose3-TissueNet on melanoma MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose3-TissueNet on melanoma MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 3. *Middle:* Pixel-based segmentation analysis by Cellpose3-TissueNet on melanoma MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose3-TissueNet applied to melanoma MIBI Image 3.

Supplementary Figure S4. Denoised, Contrast-enhanced Melanoma MIBI Images with Pixel- and Object-based Segmentations by the Cellpose3 Trained Models



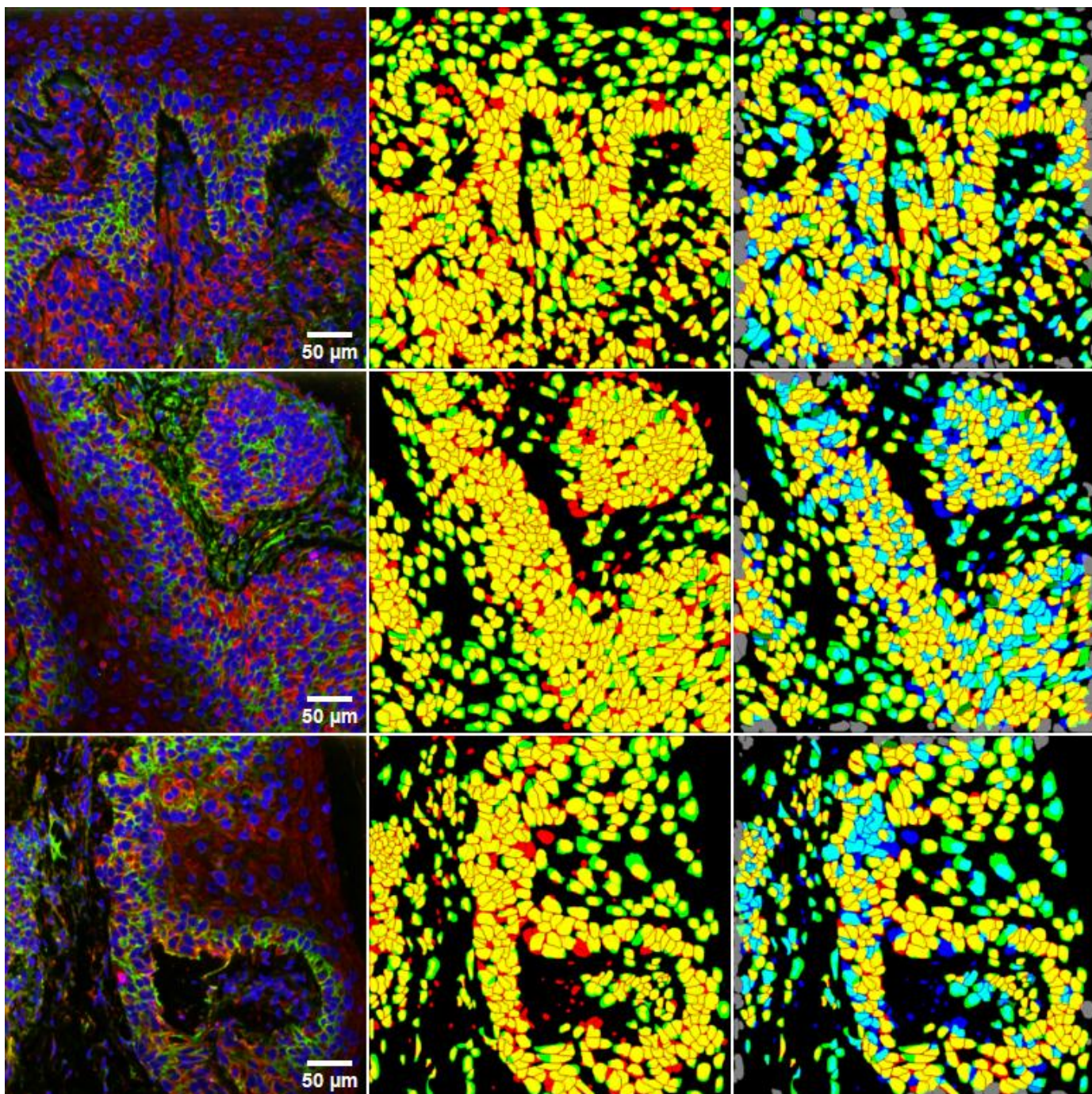
Top row: *Left:* Denoised, contrast-enhanced melanoma MIBI Image 1. *Middle:* Pixel-based segmentation analysis by the Cellpose3-trained models on melanoma MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3 trained models on melanoma MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 2. *Middle:* Pixel-based segmentation analysis by the Cellpose3 trained models on melanoma MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3 trained models on melanoma MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 3. *Middle:* Pixel-based segmentation analysis by the Cellpose3 trained models on melanoma MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3 trained models applied to melanoma MIBI Image 3.

Supplementary Figure S5. Denoised, Contrast-enhanced Melanoma MIBI Images with Pixel- and Object-based Segmentations by the Cellpose3 Models Trained with Augmented Data



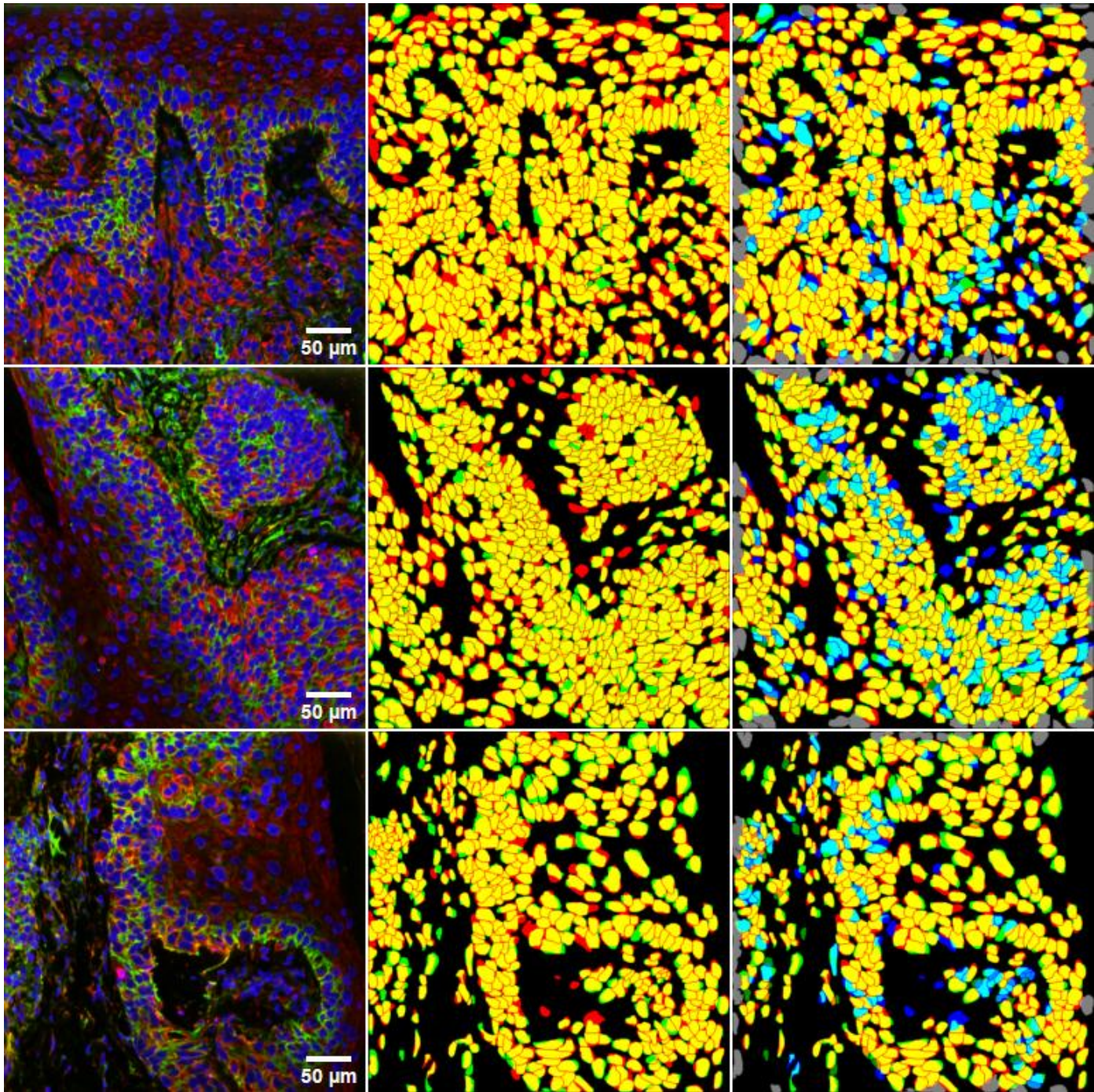
Top row: *Left:* Denoised, contrast-enhanced melanoma MIBI Image 1. *Middle:* Pixel-based segmentation analysis by the Cellpose3 models trained with augmented data on melanoma MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3 models trained with augmented data on melanoma MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 2. *Middle:* Pixel-based segmentation analysis by the Cellpose3 models trained with augmented data on melanoma MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3 models trained with augmented data on melanoma MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 3. *Middle:* Pixel-based segmentation analysis by the Cellpose3 models trained with augmented data on melanoma MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3 models trained with augmented data applied to melanoma MIBI Image 3.

Supplementary Figure S6. Denoised, Contrast-enhanced Melanoma MIBI Images with Pixel- and Object-based Segmentations by Cellpose4-SAM



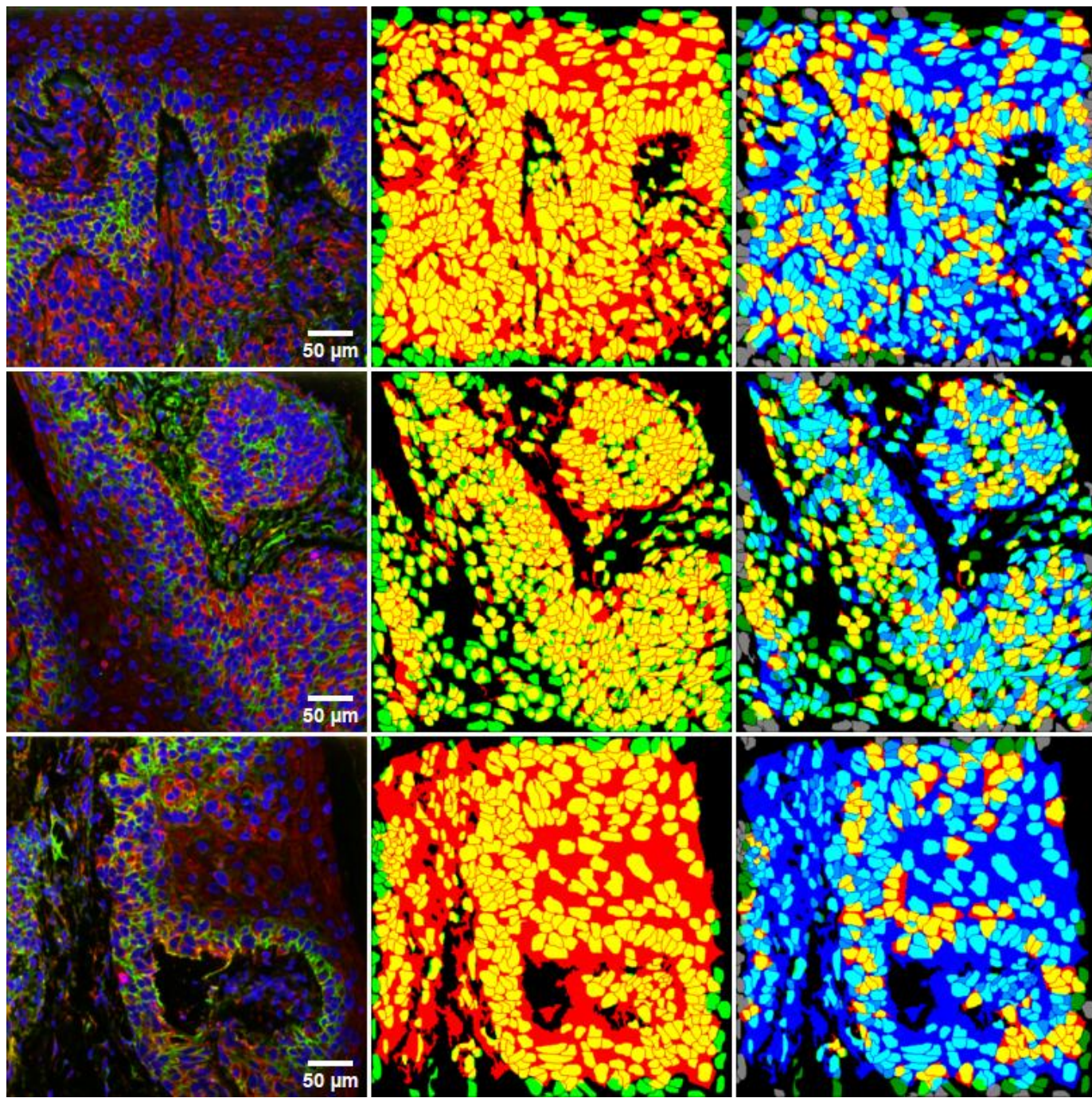
Top row: *Left:* Denoised, contrast-enhanced melanoma MIBI Image 1. *Middle:* Pixel-based segmentation analysis by Cellpose4-SAM on melanoma MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose4-SAM on melanoma MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 2. *Middle:* Pixel-based segmentation analysis by Cellpose4-SAM on melanoma MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose4-SAM on melanoma MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 3. *Middle:* Pixel-based segmentation analysis by Cellpose4-SAM on melanoma MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose4-SAM applied to melanoma MIBI Image 3.

Supplementary Figure S7. Denoised, Contrast-enhanced Melanoma MIBI Images with Pixel- and Object-based Segmentations by the Cellpose4-SAM Trained Models



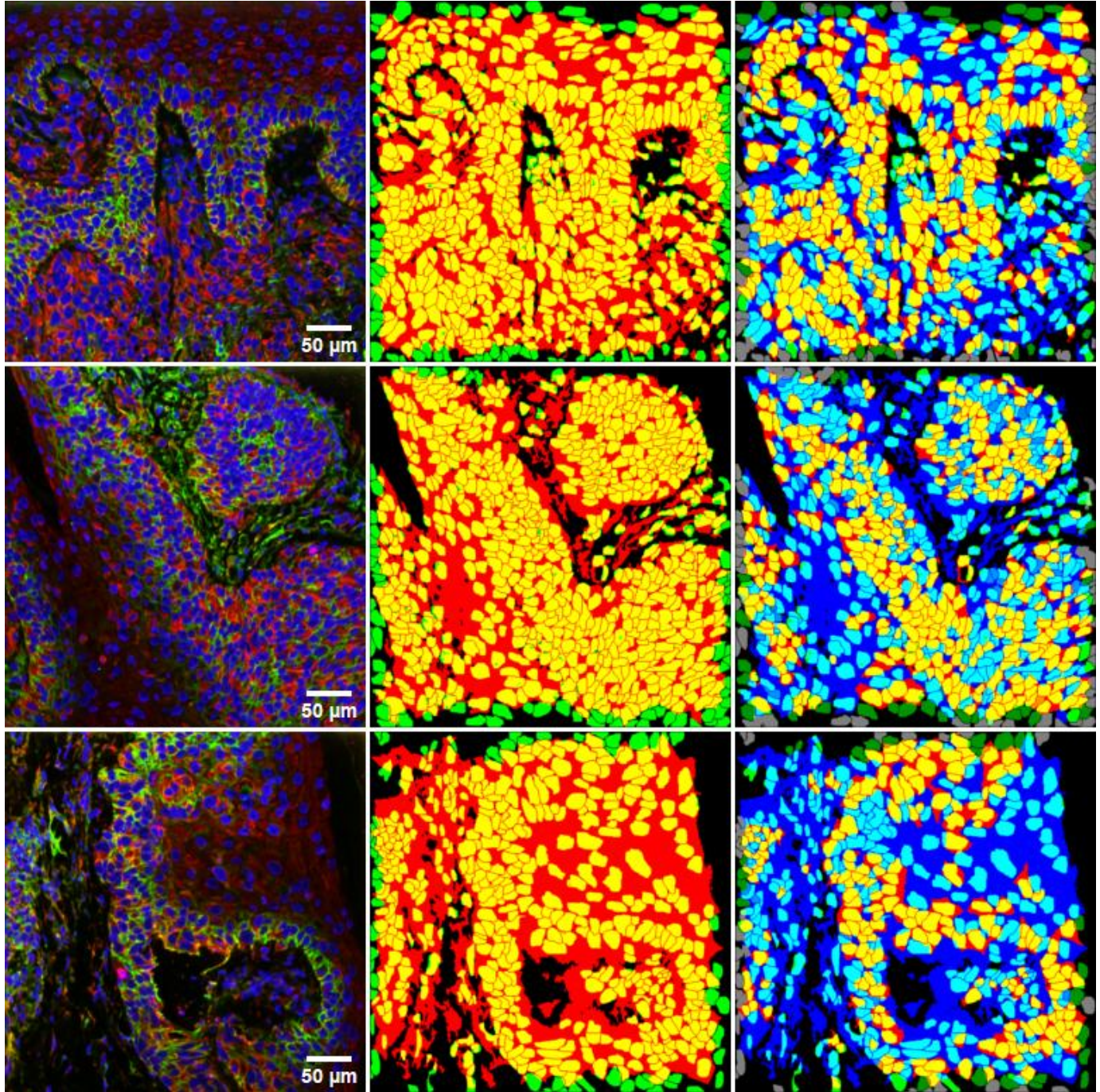
Top row: *Left:* Denoised, contrast-enhanced melanoma MIBI Image 1. *Middle:* Pixel-based segmentation analysis by the Cellpose4-SAM trained models on melanoma MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose4-SAM trained models on melanoma MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 2. *Middle:* Pixel-based segmentation analysis by the Cellpose4-SAM trained models on melanoma MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose4-SAM trained models on melanoma MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 3. *Middle:* Pixel-based segmentation analysis by the Cellpose4-SAM trained models on melanoma MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose4-SAM trained models applied to melanoma MIBI Image 3.

Supplementary Figure S8. Denoised, Contrast-enhanced Melanoma MIBI Images with Pixel- and Object-based Segmentations by CellProfiler



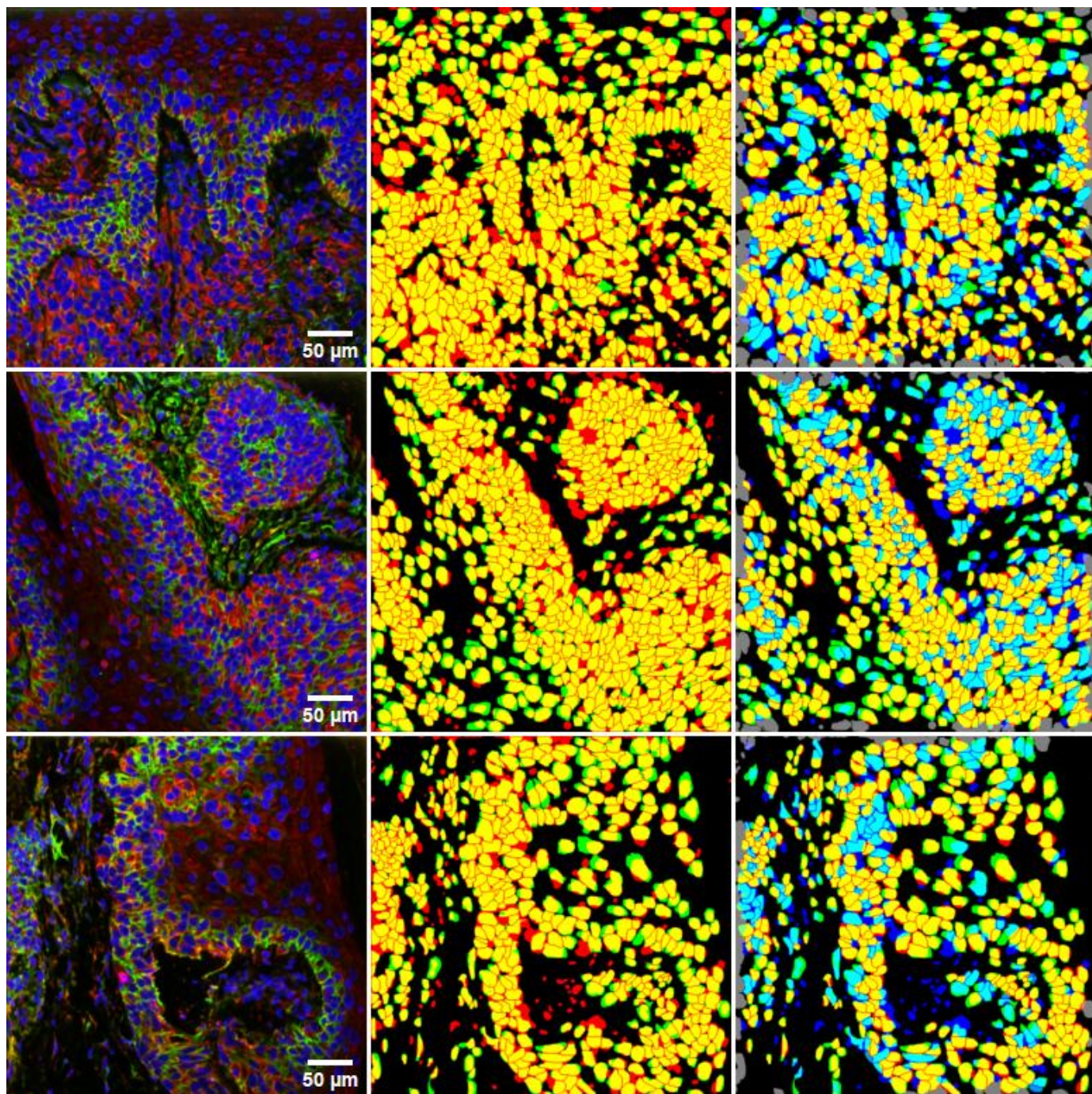
Top row: *Left:* Denoised, contrast-enhanced melanoma MIBI Image 1. *Middle:* Pixel-based segmentation analysis by CellProfiler on melanoma MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by CellProfiler on melanoma MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 2. *Middle:* Pixel-based segmentation analysis by CellProfiler on melanoma MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by CellProfiler on melanoma MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 3. *Middle:* Pixel-based segmentation analysis by CellProfiler on melanoma MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by CellProfiler applied to melanoma MIBI Image 3.

Supplementary Figure S9. Denoised, Contrast-enhanced Melanoma MIBI Images with Pixel- and Object-based Segmentations by CellProfiler-StarDist



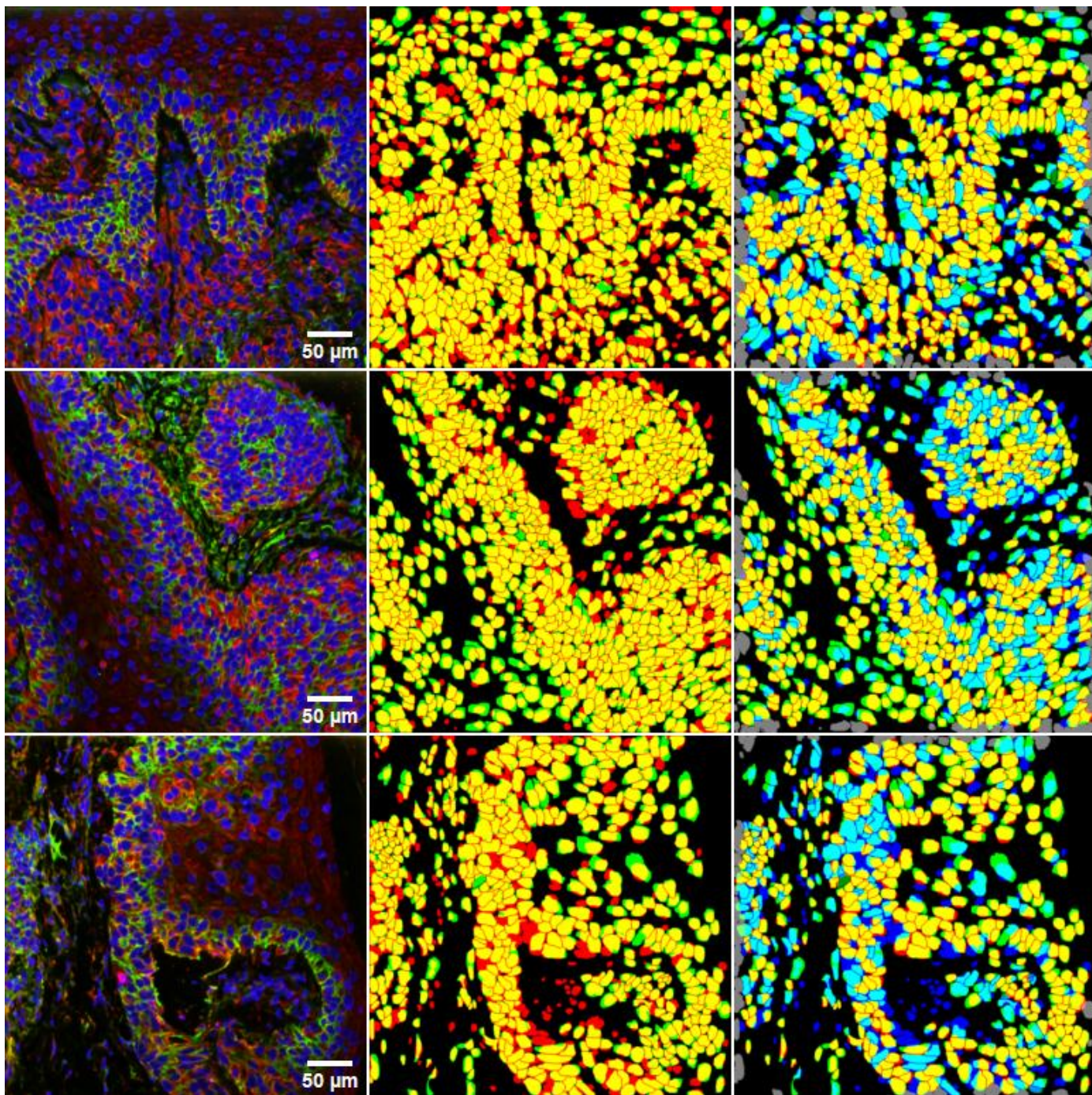
Top row: *Left:* Denoised, contrast-enhanced melanoma MIBI Image 1. *Middle:* Pixel-based segmentation analysis by CellProfiler-StarDist on melanoma MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by CellProfiler-StarDist on melanoma MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 2. *Middle:* Pixel-based segmentation analysis by CellProfiler-StarDist on melanoma MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by CellProfiler-StarDist on melanoma MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 3. *Middle:* Pixel-based segmentation analysis by CellProfiler-StarDist on melanoma MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by CellProfiler-StarDist applied to melanoma MIBI Image 3.

Supplementary Figure S10. Denoised, Contrast-enhanced Melanoma MIBI Images with Pixel- and Object-based Segmentations by Mesmer



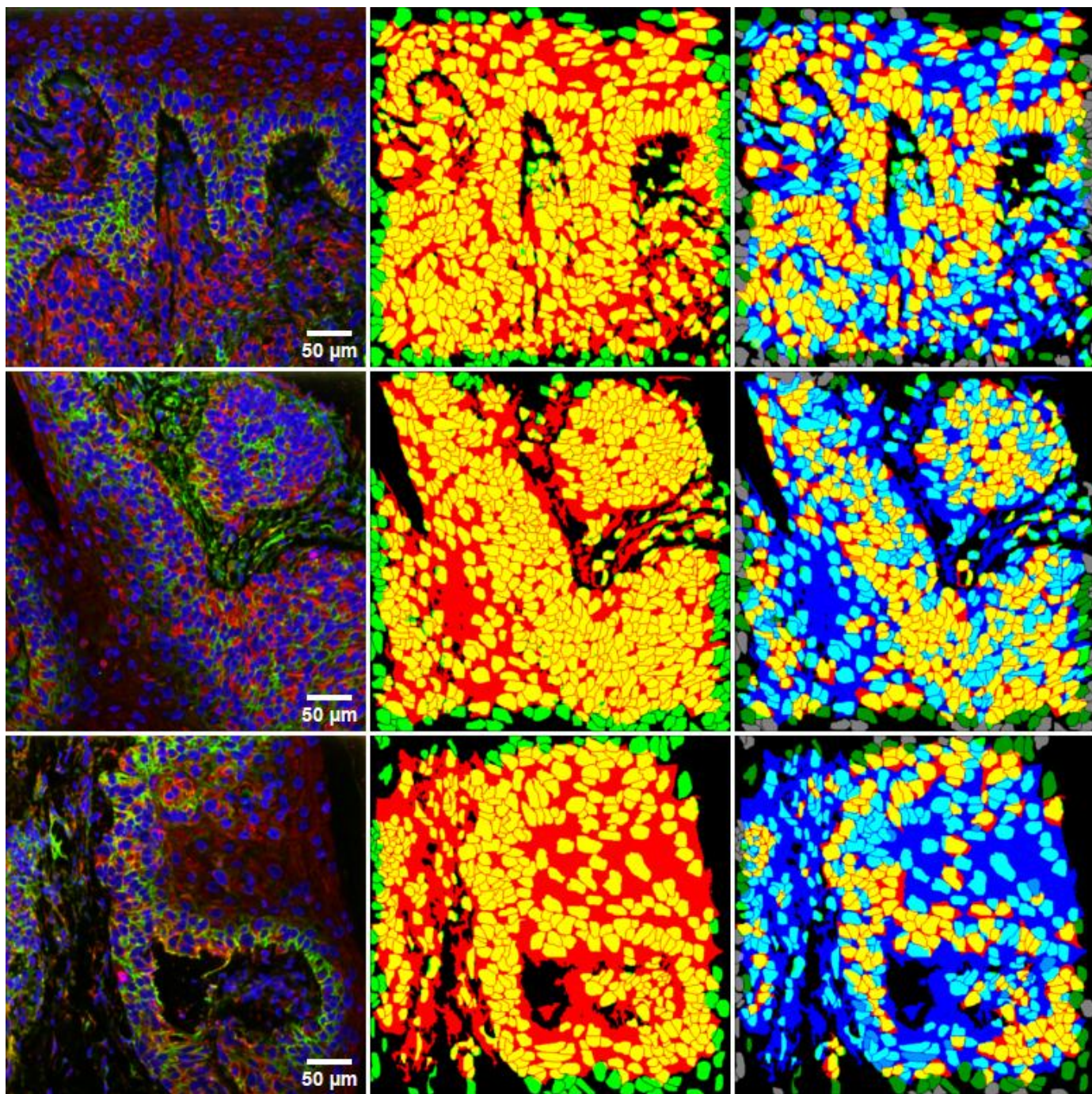
Top row: *Left:* Denoised, contrast-enhanced melanoma MIBI Image 1. *Middle:* Pixel-based segmentation analysis by Mesmer on melanoma MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by Mesmer on melanoma MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 2. *Middle:* Pixel-based segmentation analysis by Mesmer on melanoma MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by Mesmer on melanoma MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 3. *Middle:* Pixel-based segmentation analysis by Mesmer on melanoma MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by Mesmer applied to melanoma MIBI Image 3.

Supplementary Figure S11. Denoised, Contrast-enhanced Melanoma MIBI Images with Pixel- and Object-based Segmentations by napari-CellSAM



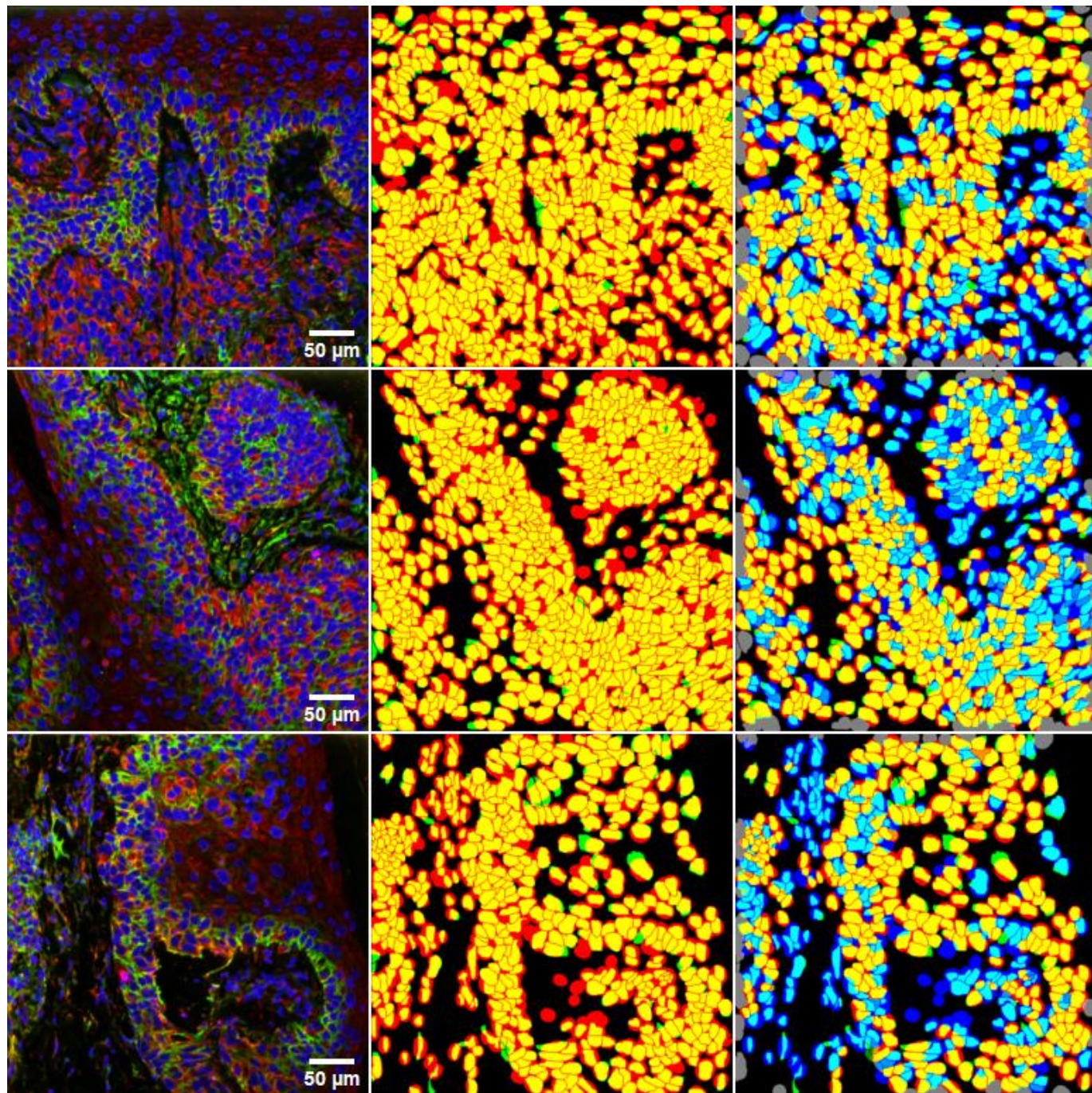
Top row: *Left:* Denoised, contrast-enhanced melanoma MIBI Image 1. *Middle:* Pixel-based segmentation analysis by napari-CellSAM on melanoma MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by napari-CellSAM on melanoma MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 2. *Middle:* Pixel-based segmentation analysis by napari-CellSAM on melanoma MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by napari-CellSAM on melanoma MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 3. *Middle:* Pixel-based segmentation analysis by napari-CellSAM on melanoma MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by napari-CellSAM applied to melanoma MIBI Image 3.

Supplementary Figure S12. Denoised, Contrast-enhanced Melanoma MIBI Images with Pixel- and Object-based Segmentations by QuPath



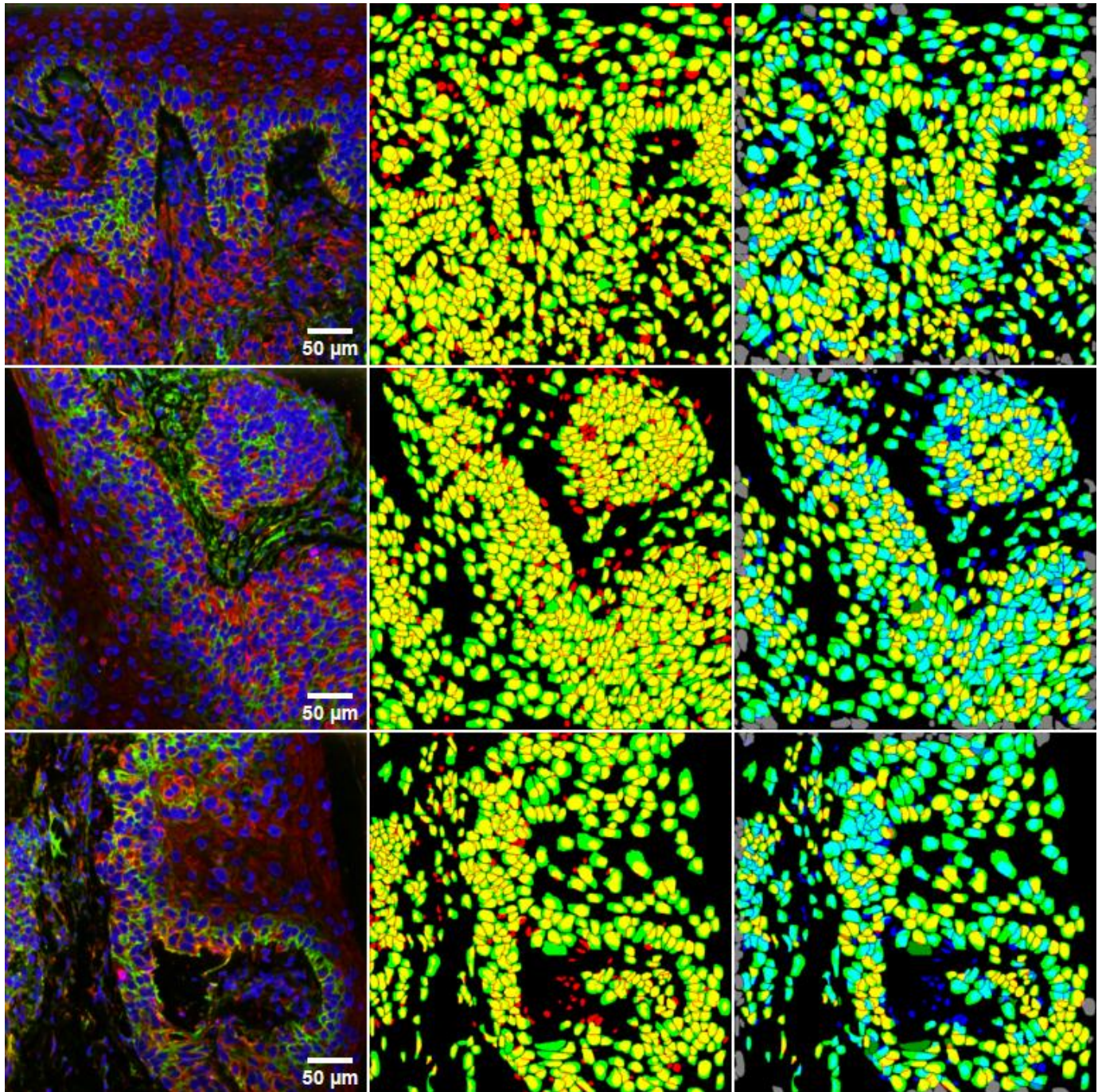
Top row: *Left:* Denoised, contrast-enhanced melanoma MIBI Image 1. *Middle:* Pixel-based segmentation analysis (IoU=0.5) by QuPath on melanoma MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath on melanoma MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 2. *Middle:* Pixel-based segmentation analysis by QuPath on melanoma MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath on melanoma MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 3. *Middle:* Pixel-based segmentation analysis by QuPath on melanoma MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath applied to melanoma MIBI Image 3.

Supplementary Figure S13. Denoised, Contrast-enhanced Melanoma MIBI Images with Pixel- and Object-based Segmentations by QuPath-StarDist



Top row: *Left:* Denoised, contrast-enhanced melanoma MIBI Image 1. *Middle:* Pixel-based segmentation analysis (IoU=0.5) by QuPath-StarDist on melanoma MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath-StarDist on melanoma MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 2. *Middle:* Pixel-based segmentation analysis by QuPath-StarDist on melanoma MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath-StarDist on melanoma MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 3. *Middle:* Pixel-based segmentation analysis by QuPath-StarDist on melanoma MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath-StarDist applied to melanoma MIBI Image 3.

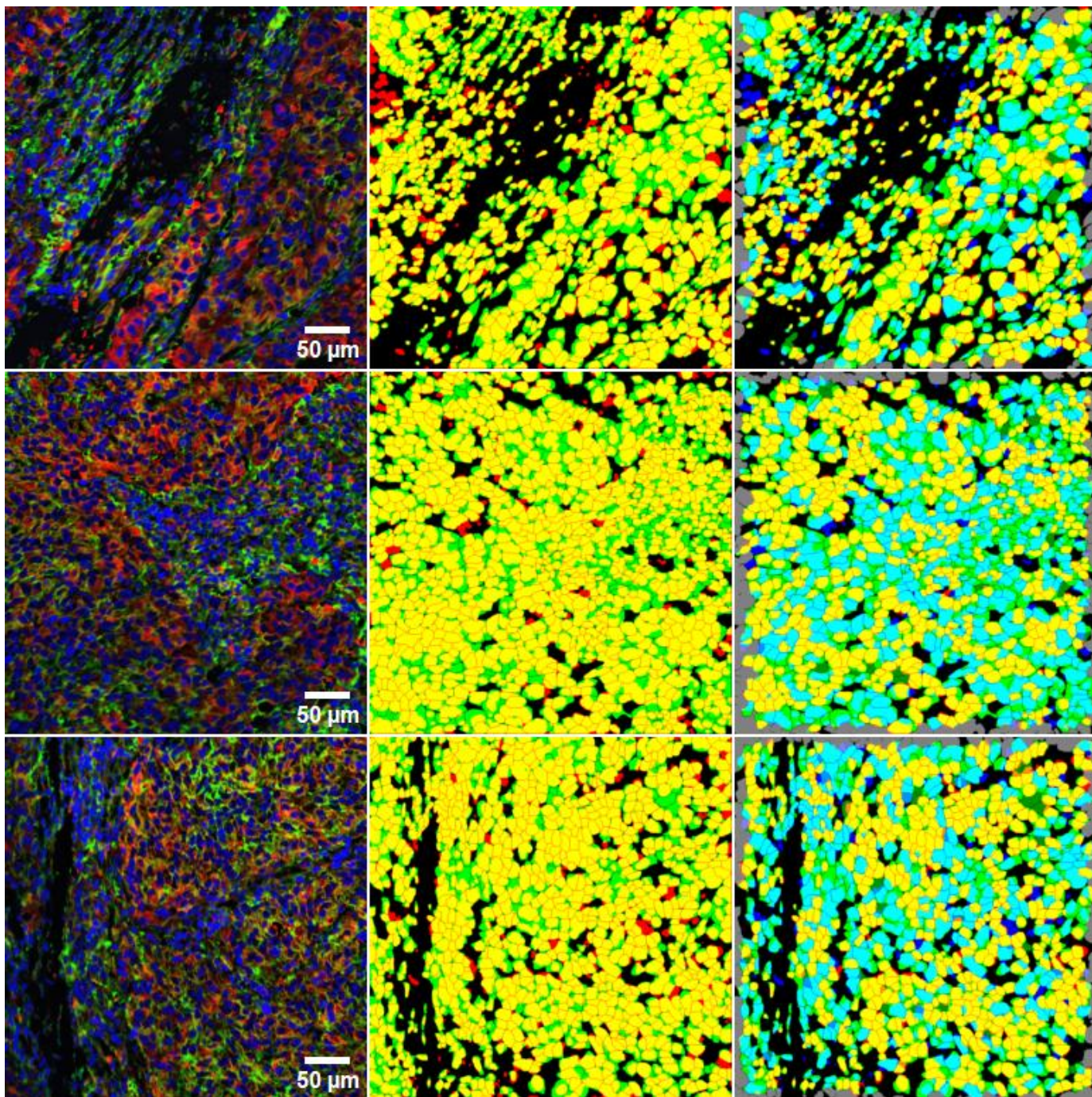
Supplementary Figure S14. Denoised, Contrast-enhanced Melanoma MIBI Images with Pixel- and Object-based Segmentations by QuPath-InstanSeg



Top row: *Left:* Denoised, contrast-enhanced melanoma MIBI Image 1. *Middle:* Pixel-based segmentation analysis by QuPath-InstanSeg on melanoma MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath-InstanSeg on melanoma MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 2. *Middle:* Pixel-based segmentation analysis by QuPath-InstanSeg on melanoma MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath-InstanSeg on melanoma MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced melanoma MIBI Image 3. *Middle:* Pixel-based segmentation analysis by QuPath-InstanSeg on melanoma MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath-InstanSeg applied to melanoma MIBI Image 3.

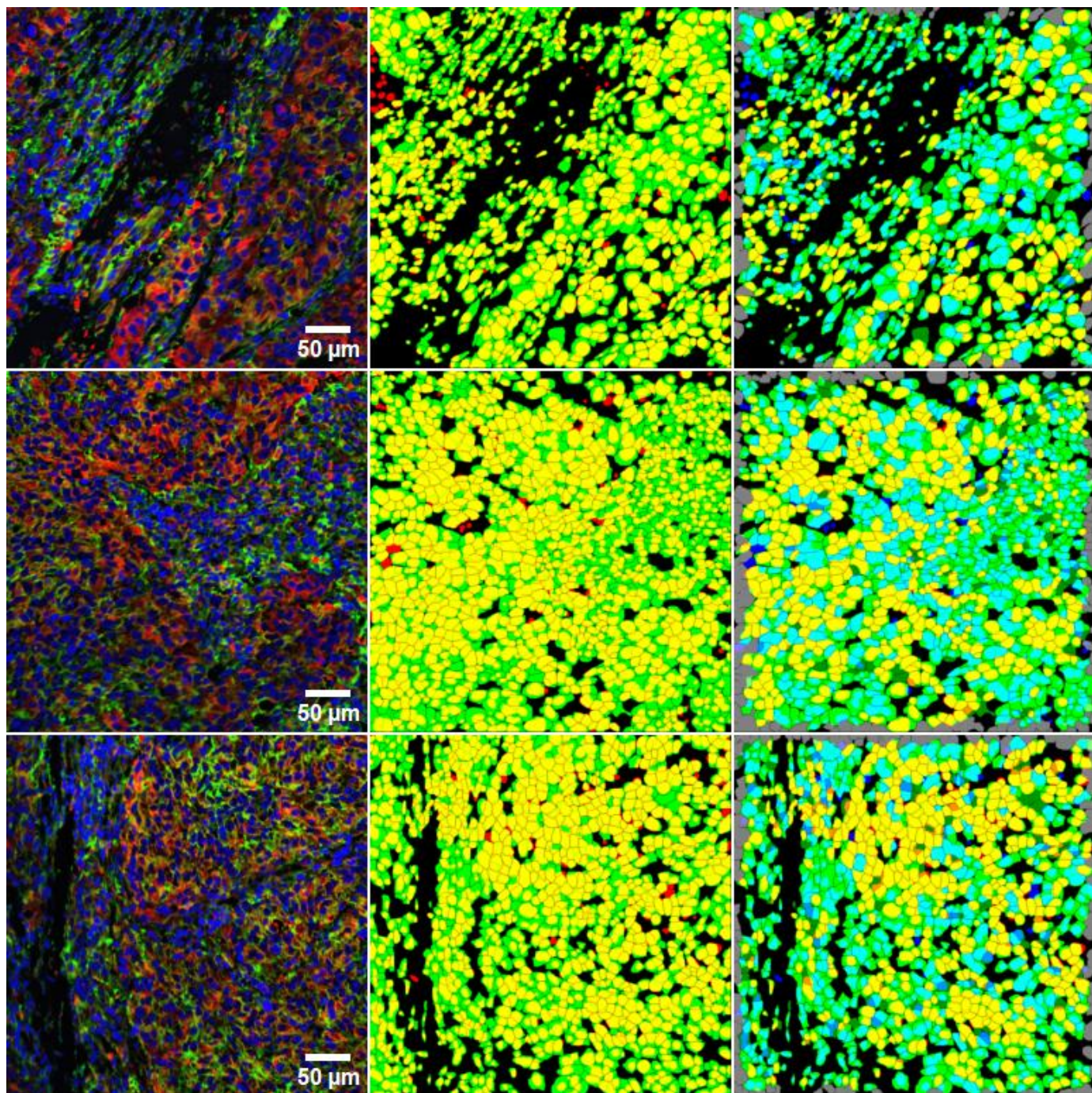
Supplementary Figures for Breast Cancer MIBI Images

Supplementary Figure S15. Denoised, Contrast-enhanced Breast Cancer MIBI Images with Pixel- and Object-based Segmentations by the Cellpose3-Cyto3 Model



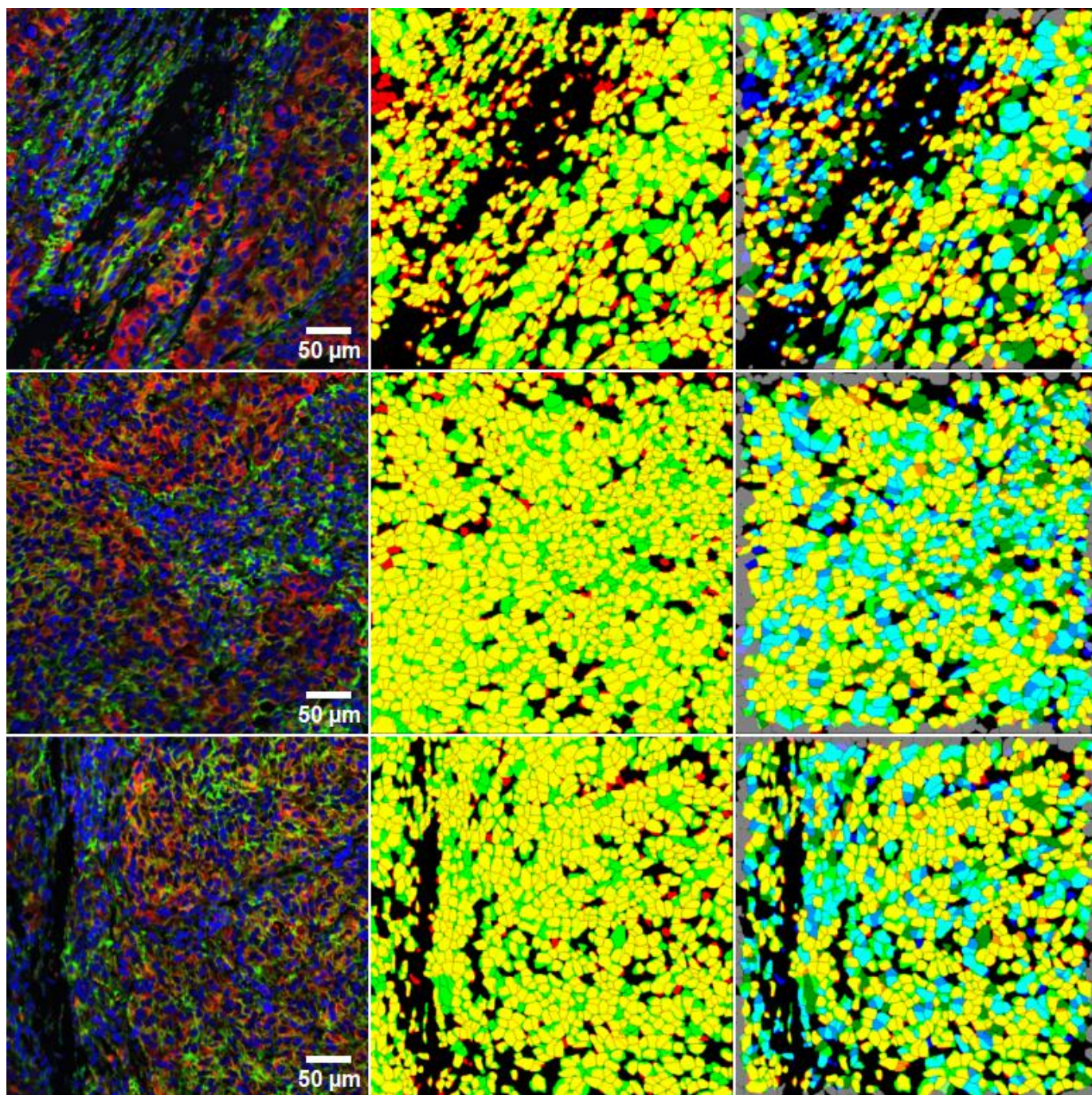
Top row: *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 1. *Middle:* Pixel-based segmentation analysis by Cellpose3-Cyto3 model on breast cancer MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose3-Cyto3 model on breast cancer MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 2. *Middle:* Pixel-based segmentation analysis by Cellpose3-Cyto3 model on breast cancer MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose3-Cyto3 model on breast cancer MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 3. *Middle:* Pixel-based segmentation analysis by Cellpose3-Cyto3 model on breast cancer MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose3-Cyto3 model applied to breast cancer MIBI Image 3.

Supplementary Figure S16. Denoised, Contrast-enhanced Breast Cancer MIBI Images with Pixel- and Object-based Segmentations by the Cellpose3-Tissue Net Model



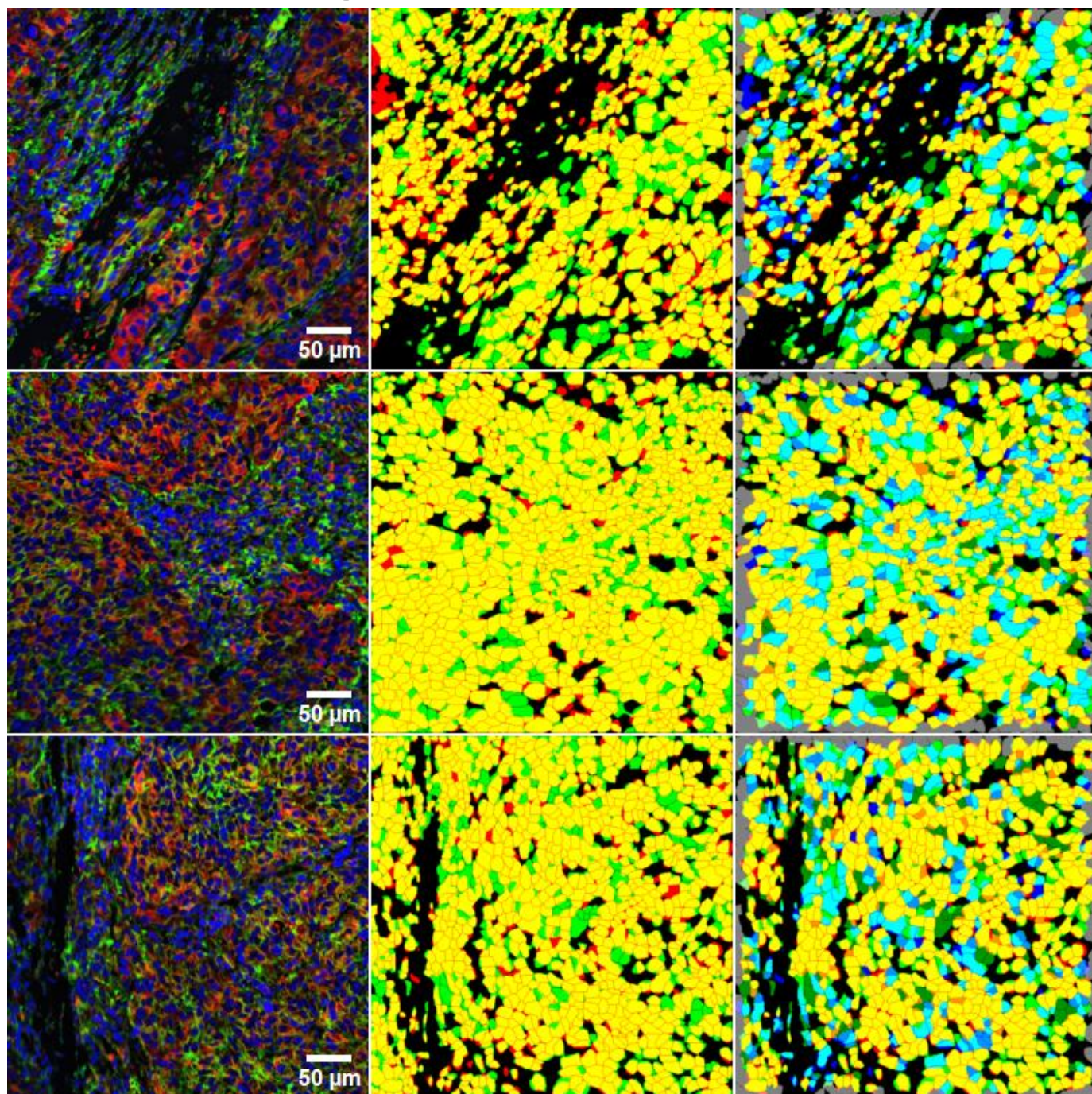
Top row: *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 1. *Middle:* Pixel-based segmentation analysis by the Cellpose3-Tissue Net model on breast cancer MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3-Tissue Net model on breast cancer MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 2. *Middle:* Pixel-based segmentation analysis by the Cellpose3-Tissue Net model on breast cancer MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3-Tissue Net model on breast cancer MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 3. *Middle:* Pixel-based segmentation analysis by the Cellpose3-Tissue Net model on breast cancer MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3-Tissue Net model applied to breast cancer MIBI Image 3.

Supplementary Figure S17. Denoised, Contrast-enhanced Breast Cancer MIBI Images with Pixel- and Object-based Segmentations by the Cellpose3 Trained Models



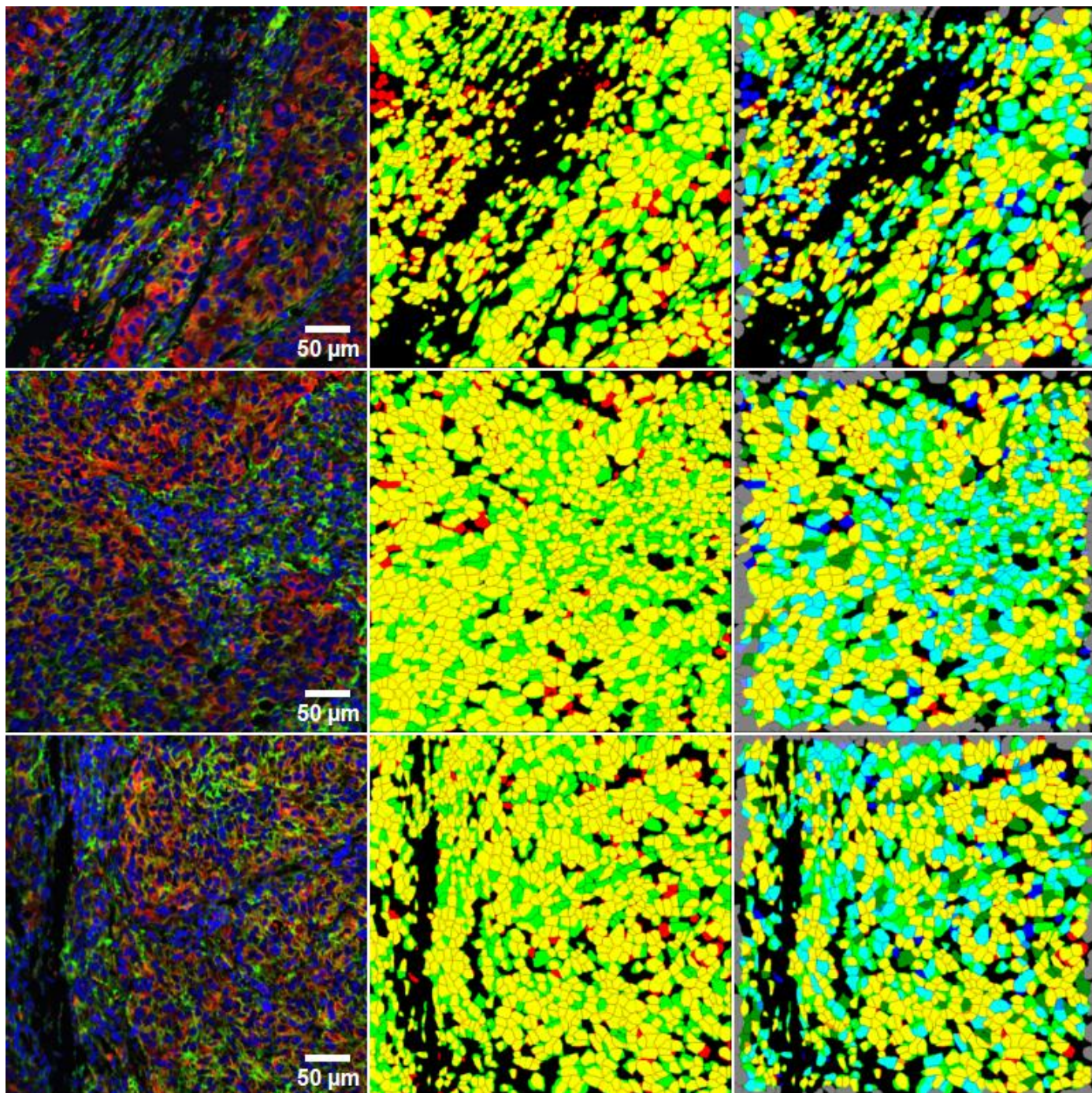
Top row: *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 1. *Middle:* Pixel-based segmentation analysis by the Cellpose3 trained models on breast cancer MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3 trained models on breast cancer MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 2. *Middle:* Pixel-based segmentation analysis by the Cellpose3 trained models on breast cancer MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3 trained models on breast cancer MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 3. *Middle:* Pixel-based segmentation analysis by the Cellpose3 trained models on breast cancer MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3 trained models applied to breast cancer MIBI Image 3.

Supplementary Figure S18. Denoised, Contrast-enhanced Breast Cancer MIBI Images with Pixel- and Object-based Segmentations by the Cellpose3 Models Trained with Augmented Data



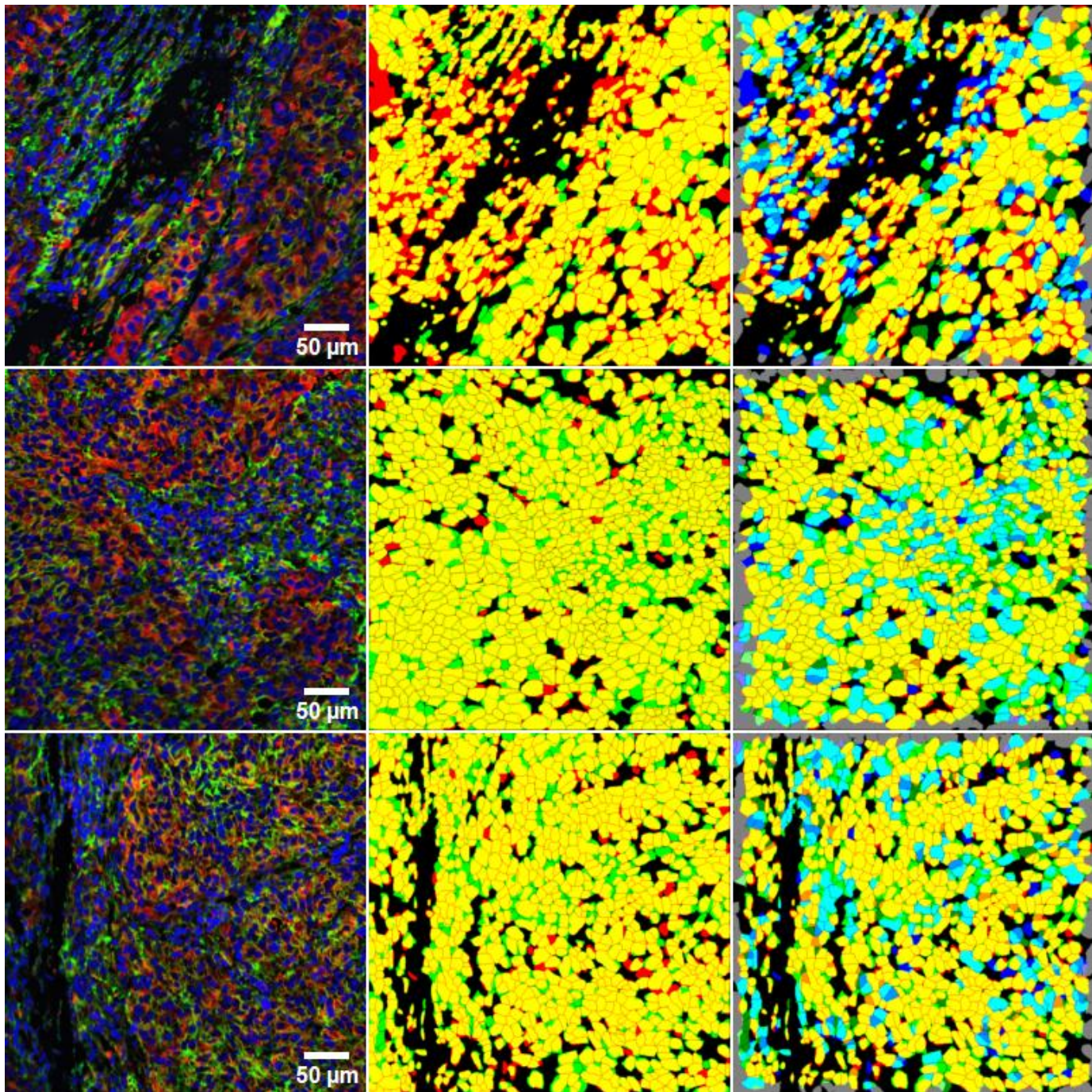
Top row: *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 1. *Middle:* Pixel-based segmentation analysis by the Cellpose3 models trained with augmented data on breast cancer MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3 models trained with augmented data on breast cancer MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 2. *Middle:* Pixel-based segmentation analysis by the Cellpose3 models trained with augmented data on breast cancer MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3 models trained with augmented data on breast cancer MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 3. *Middle:* Pixel-based segmentation analysis by the Cellpose3 models trained with augmented data on breast cancer MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3 models trained with augmented data applied to breast cancer MIBI Image 3.

Supplementary Figure S19. Denoised, Contrast-enhanced Breast Cancer MIBI Images with Pixel- and Object-based Segmentations by Cellpose4-SAM



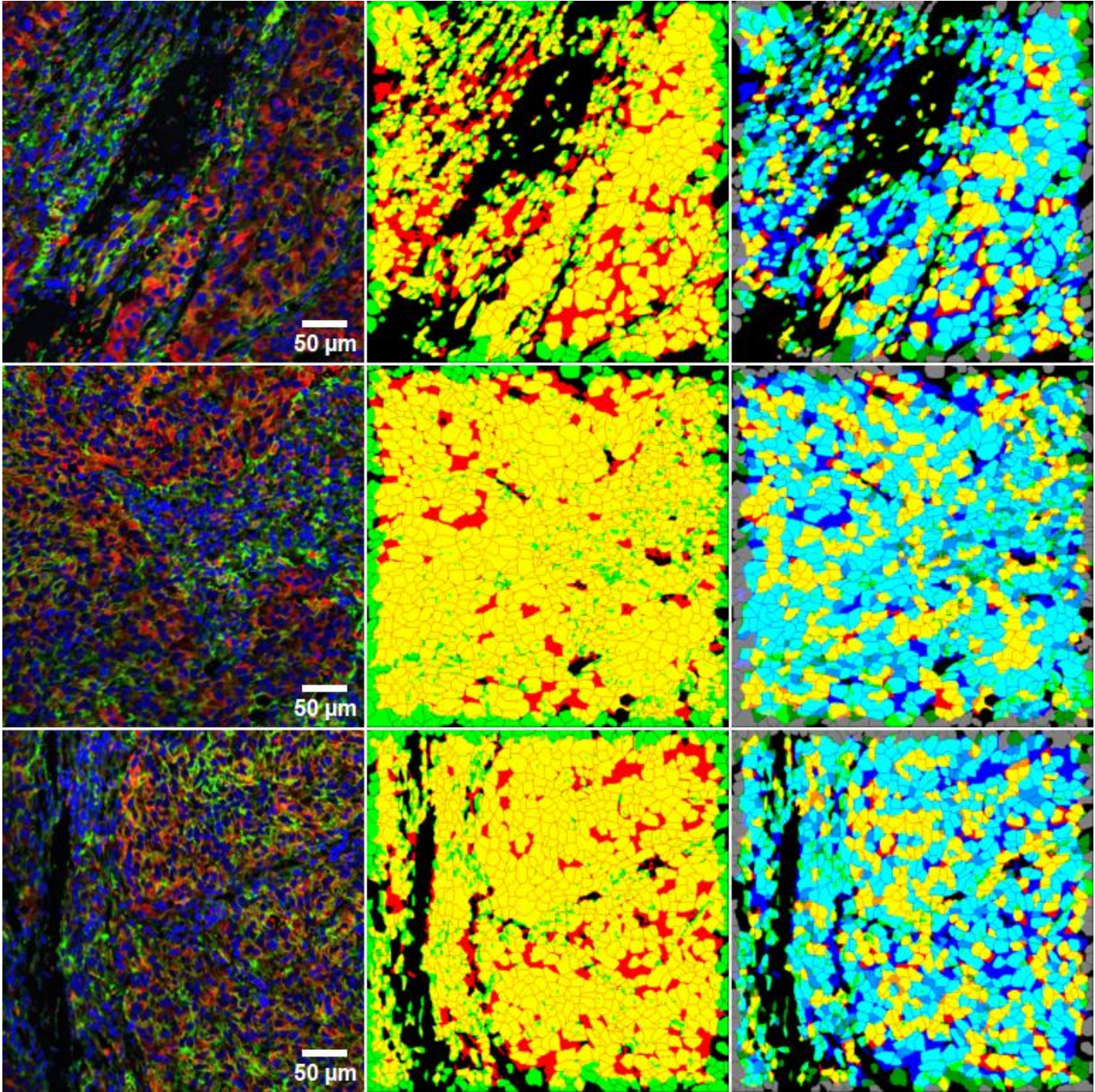
Top row: *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 1. *Middle:* Pixel-based segmentation analysis by Cellpose4-SAM on breast cancer MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose4-SAM on breast cancer MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 2. *Middle:* Pixel-based segmentation analysis by Cellpose4-SAM on breast cancer MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose4-SAM on breast cancer MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 3. *Middle:* Pixel-based segmentation analysis by Cellpose4-SAM on breast cancer MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose4-SAM applied to breast cancer MIBI Image 3.

Supplementary Figure S20. Denoised, Contrast-enhanced Breast Cancer MIBI Images with Pixel- and Object-based Segmentations by Cellpose4-SAM Trained Models



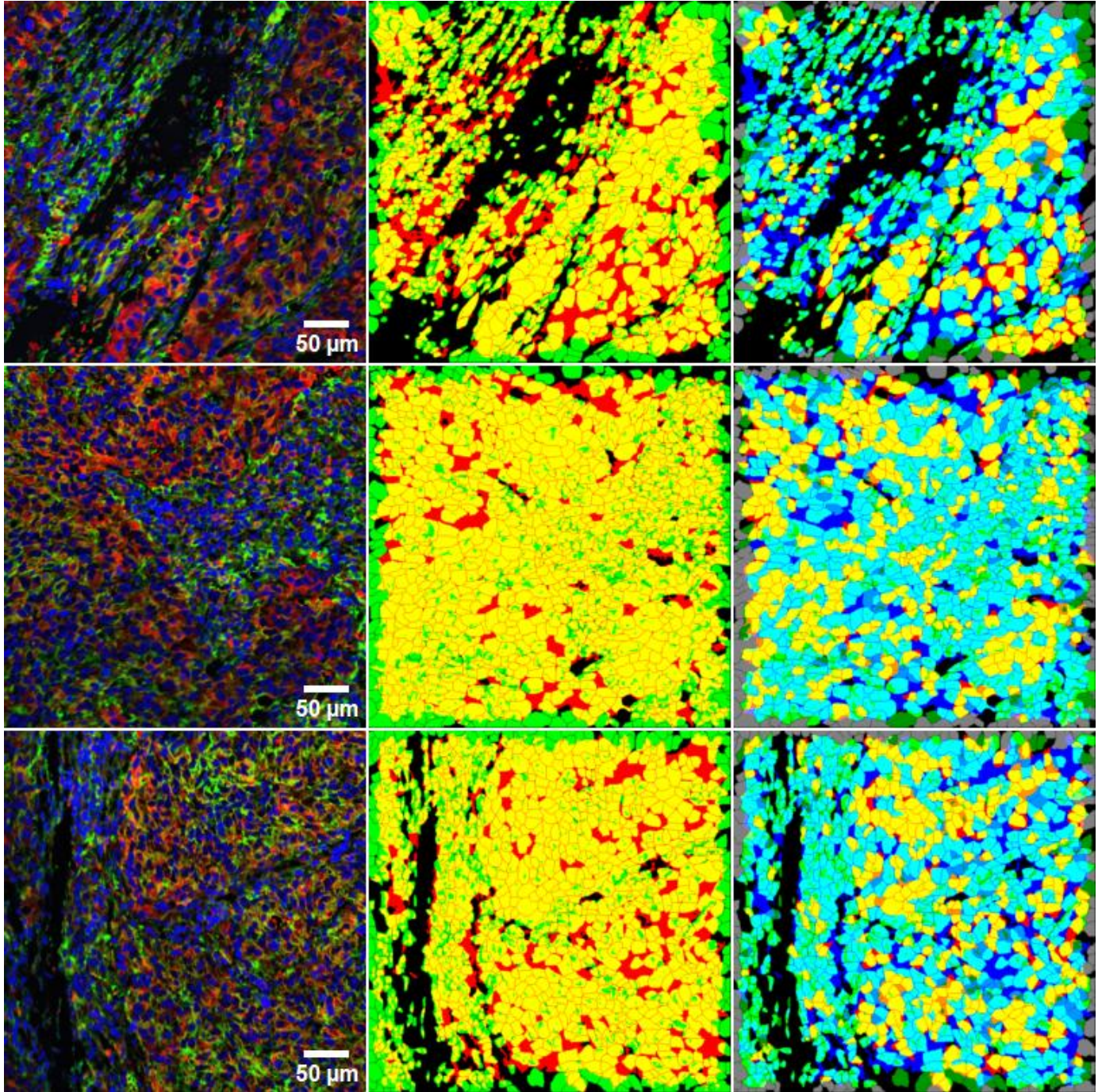
Top row: *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 1. *Middle:* Pixel-based segmentation analysis by Cellpose4-SAM trained models on breast cancer MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose4-SAM trained models on breast cancer MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 2. *Middle:* Pixel-based segmentation analysis by Cellpose4-SAM trained models on breast cancer MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose4-SAM trained models on breast cancer MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 3. *Middle:* Pixel-based segmentation analysis by Cellpose4-SAM trained models on breast cancer MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose4-SAM trained models applied to breast cancer MIBI Image 3.

Supplementary Figure S21. Denoised, Contrast-enhanced Breast Cancer MIBI Images with Pixel- and Object-based Segmentations by CellProfiler



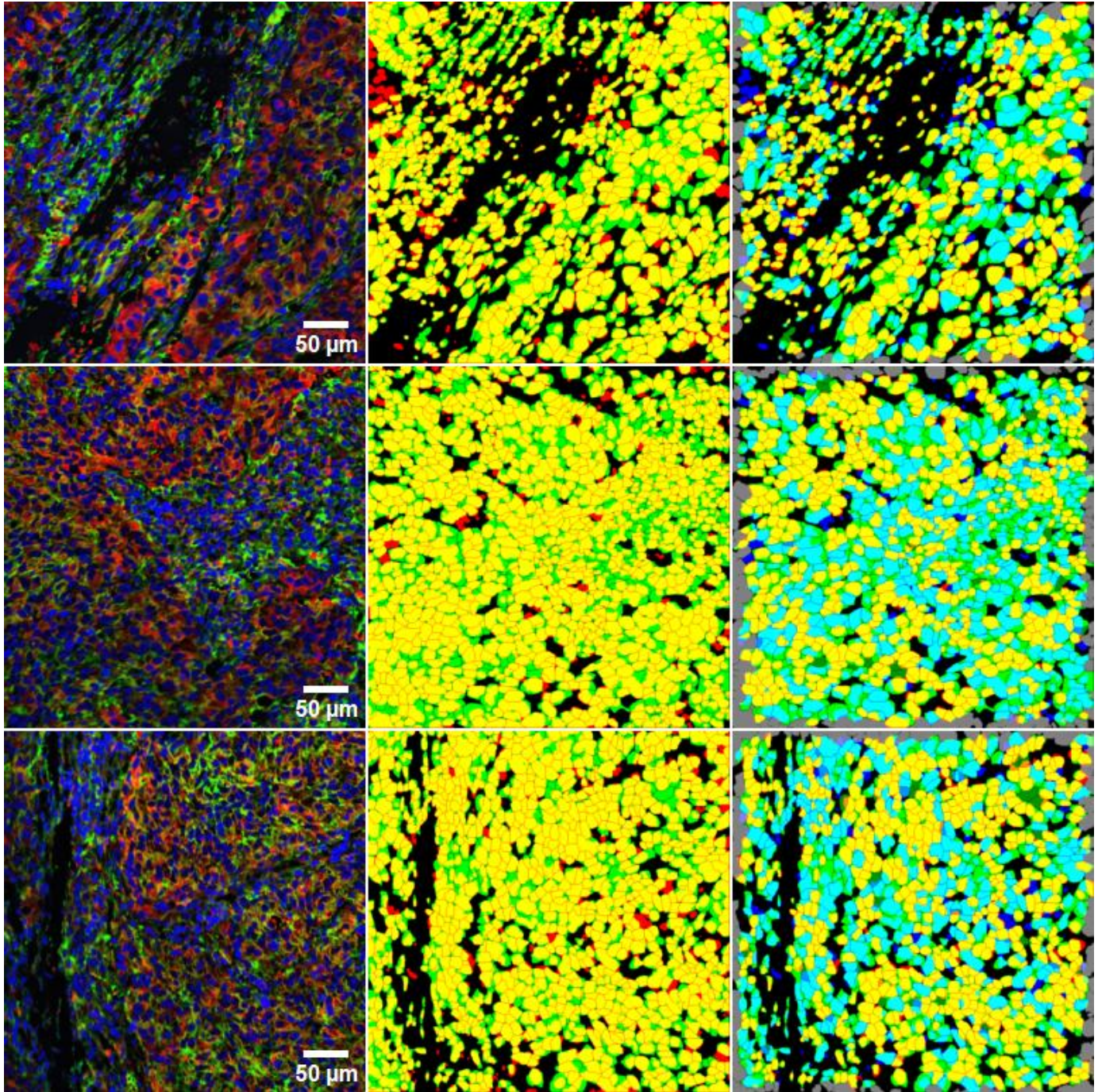
Top row: *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 1. *Middle:* Pixel-based segmentation analysis by CellProfiler on breast cancer MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by CellProfiler on breast cancer MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 2. *Middle:* Pixel-based segmentation analysis by CellProfiler on breast cancer MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by CellProfiler on breast cancer MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 3. *Middle:* Pixel-based segmentation analysis by CellProfiler on breast cancer MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by CellProfiler applied to breast cancer MIBI Image 3.

Supplementary Figure S22. Denoised, Contrast-enhanced Breast Cancer MIBI Images with Pixel- and Object-based Segmentations by CellProfiler-StarDist



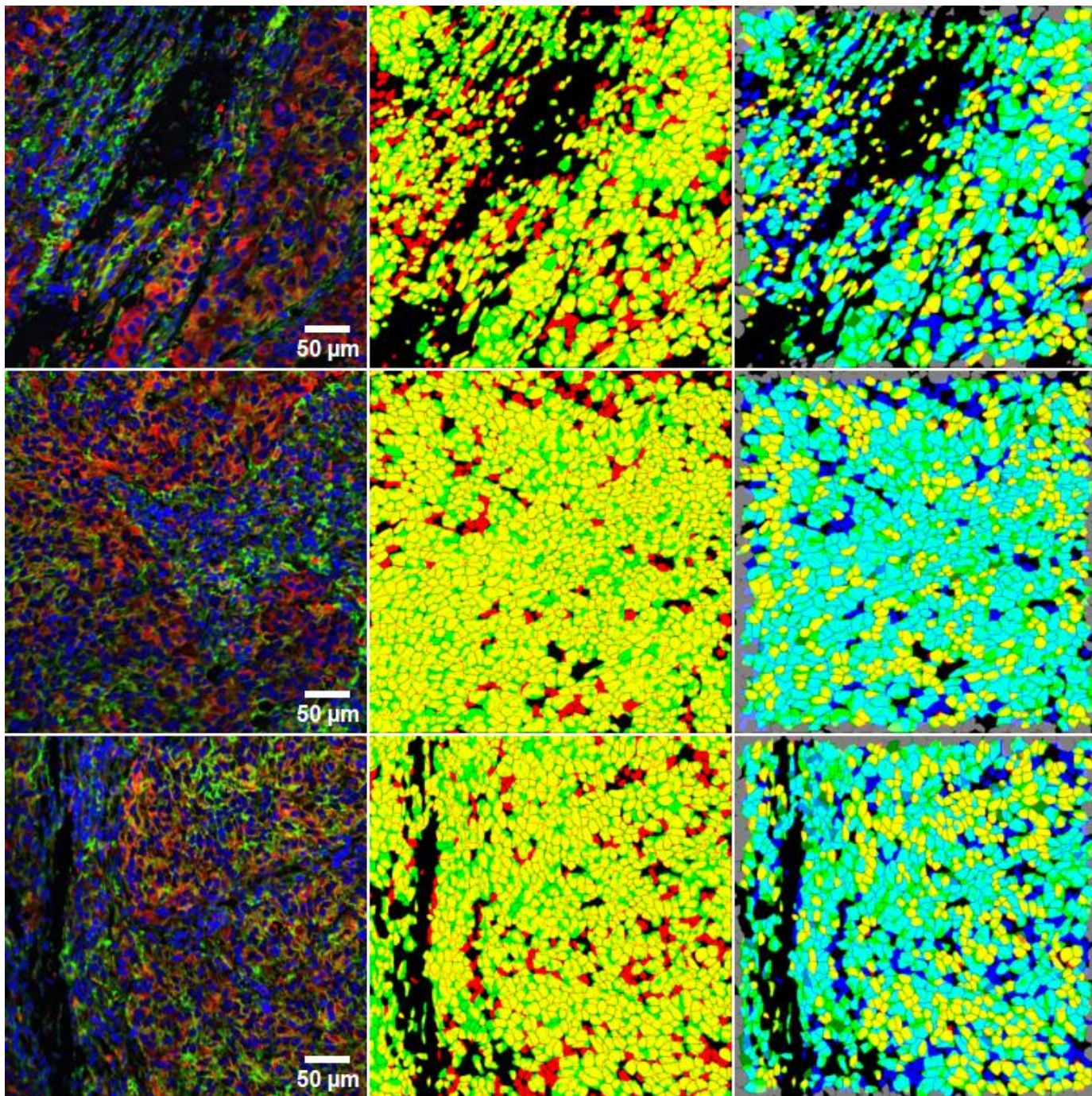
Top row: *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 1. *Middle:* Pixel-based segmentation analysis by CellProfiler-StarDist on breast cancer MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by CellProfiler-StarDist on breast cancer MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 2. *Middle:* Pixel-based segmentation analysis by CellProfiler-StarDist on breast cancer MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by CellProfiler-StarDist on breast cancer MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 3. *Middle:* Pixel-based segmentation analysis by CellProfiler-StarDist on breast cancer MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by CellProfiler-StarDist applied to breast cancer MIBI Image 3.

Supplementary Figure S23. Denoised, Contrast-enhanced Breast Cancer MIBI Images with Pixel- and Object-based Segmentations by Mesmer



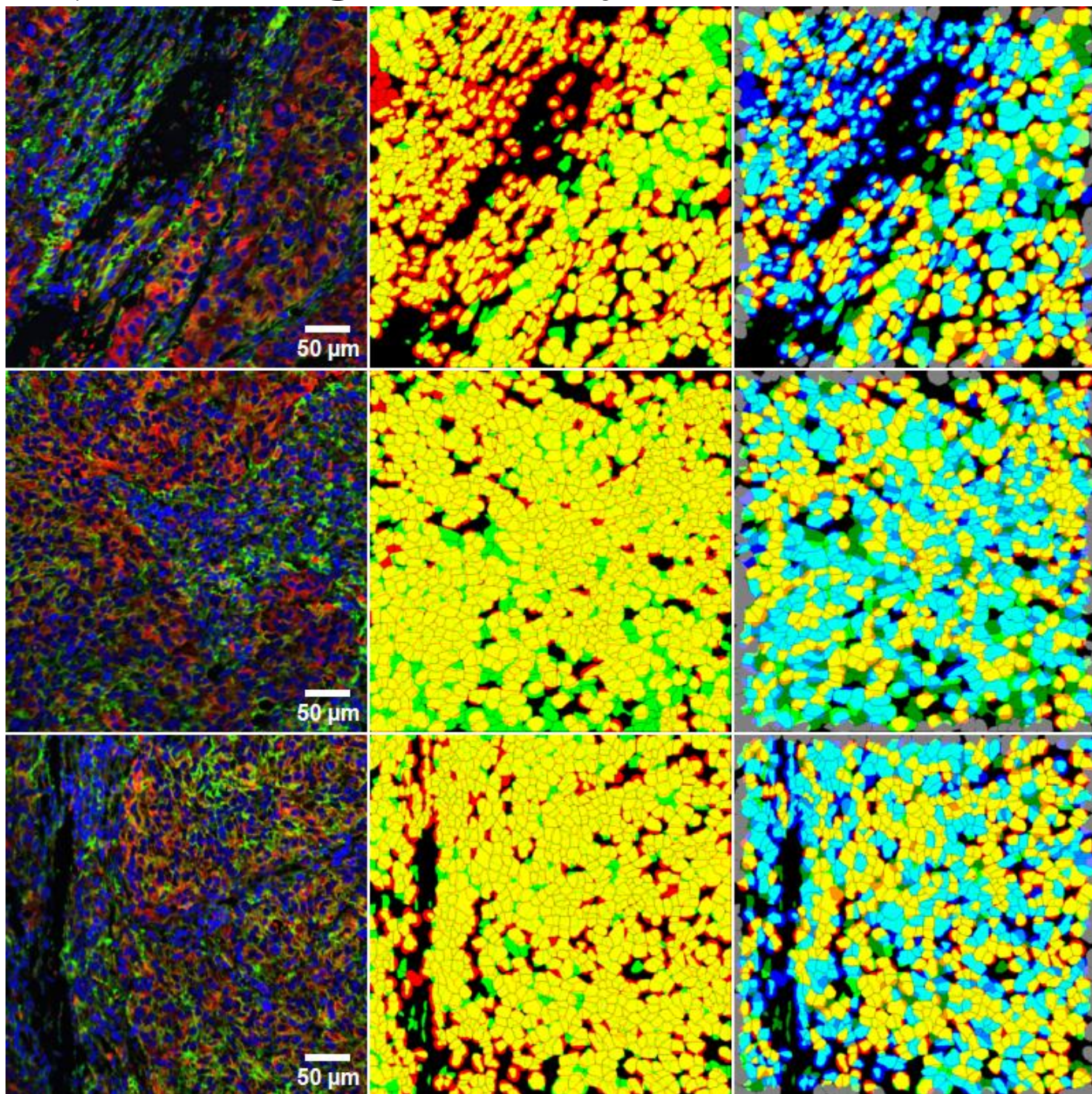
Top row: *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 1. *Middle:* Pixel-based segmentation analysis by Mesmer on breast cancer MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by Mesmer on breast cancer MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 2. *Middle:* Pixel-based segmentation analysis by Mesmer on breast cancer MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by Mesmer on breast cancer MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 3. *Middle:* Pixel-based segmentation analysis by Mesmer on breast cancer MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by Mesmer applied to breast cancer MIBI Image 3.

Supplementary Figure S24. Denoised, Contrast-enhanced Breast Cancer MIBI Images with Pixel- and Object-based Segmentations by napari-CellSAM



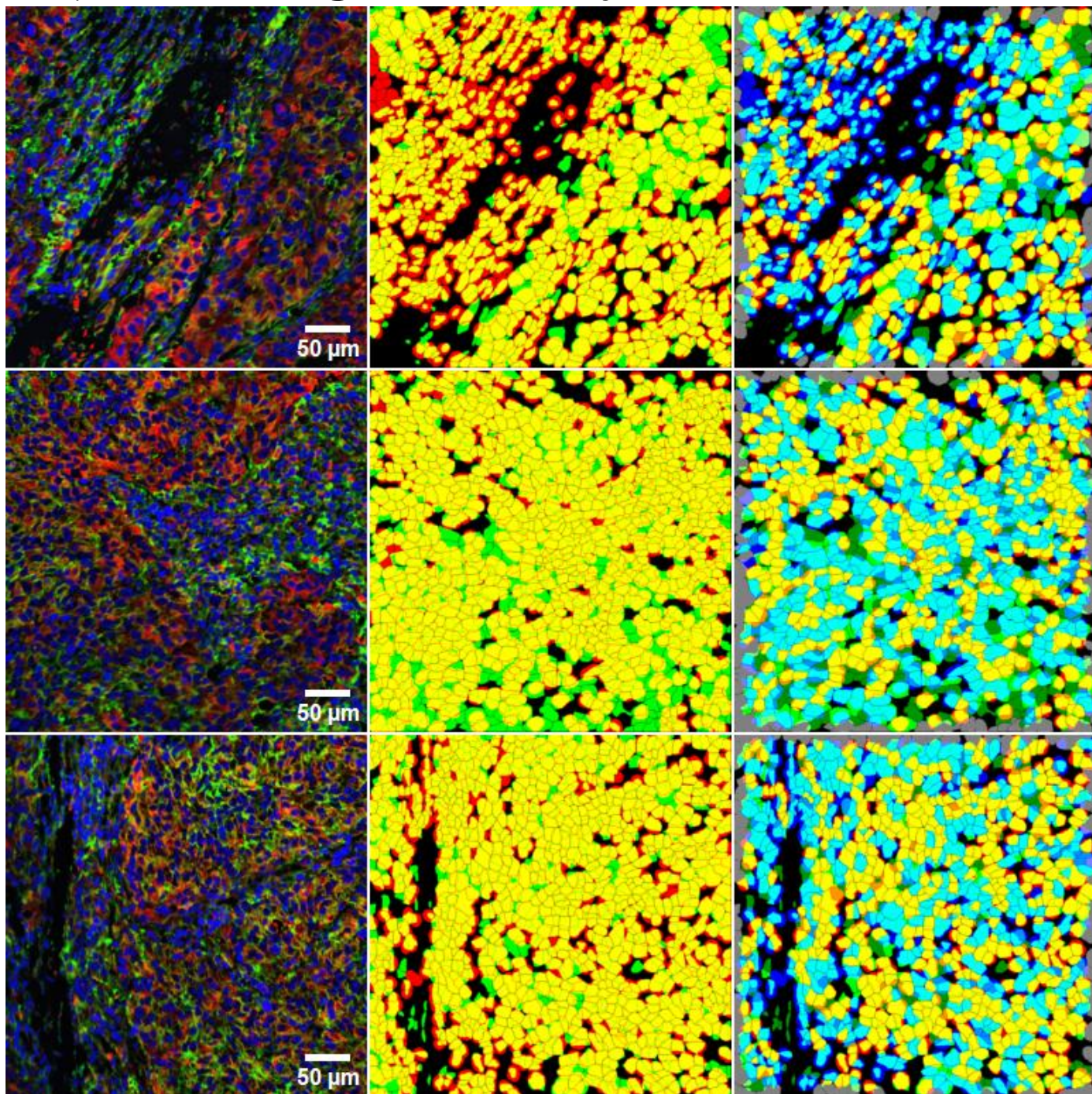
Top row: *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 1. *Middle:* Pixel-based segmentation analysis by napari-CellSAM on breast cancer MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by napari-CellSAM on breast cancer MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 2. *Middle:* Pixel-based segmentation analysis by napari-CellSAM on breast cancer MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by napari-CellSAM on breast cancer MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 3. *Middle:* Pixel-based segmentation analysis by napari-CellSAM on breast cancer MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by napari-CellSAM applied to breast cancer MIBI Image 3.

Supplementary Figure S25. Denoised, Contrast-enhanced Breast Cancer MIBI Images with Pixel- and Object-based Segmentations by QuPath



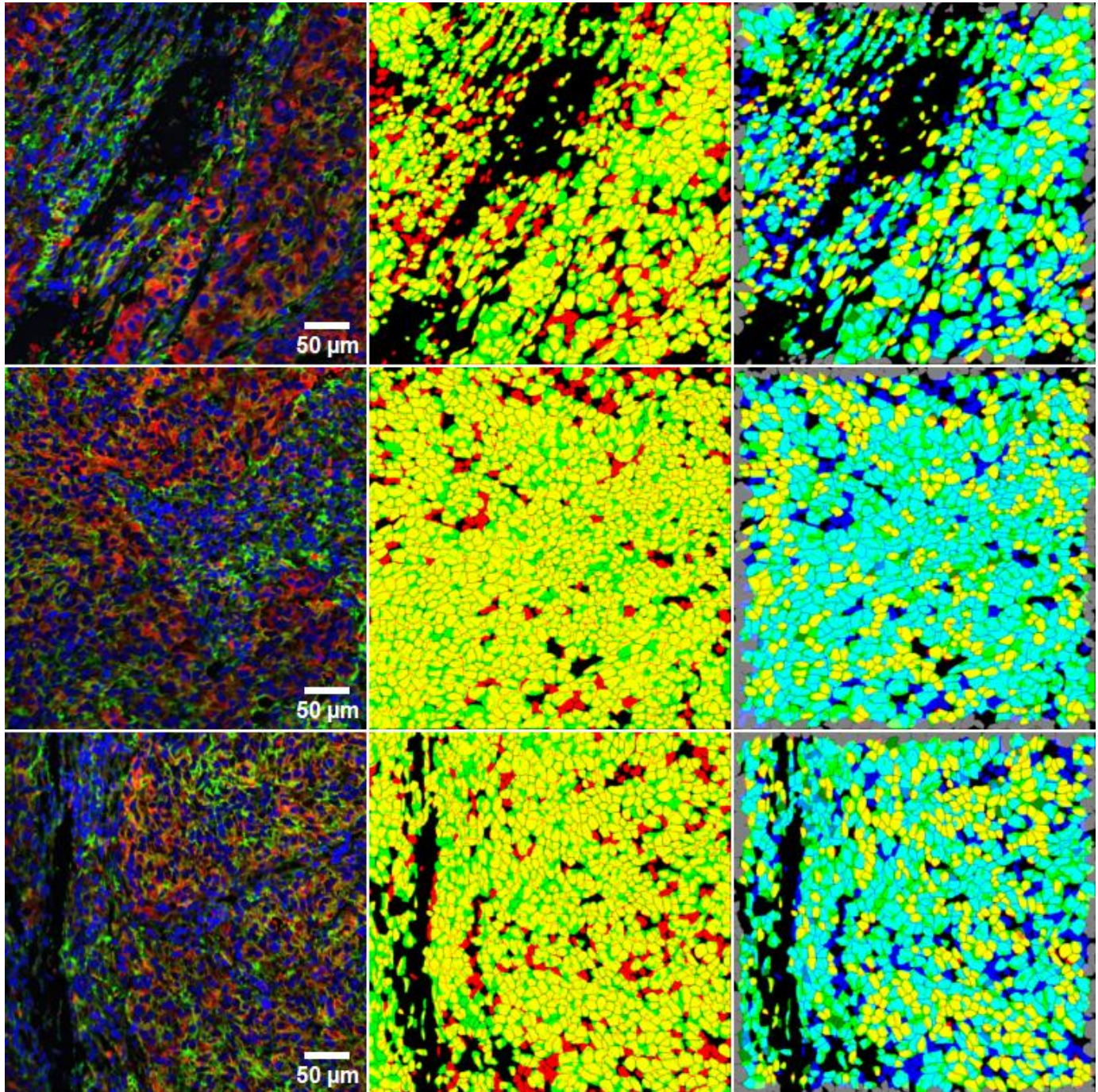
Top row: *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 1. *Middle:* Pixel-based segmentation analysis by QuPath on breast cancer MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath on breast cancer MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 2. *Middle:* Pixel-based segmentation analysis by QuPath on breast cancer MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath on breast cancer MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 3. *Middle:* Pixel-based segmentation analysis by QuPath on breast cancer MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath applied to breast cancer MIBI Image 3.

Supplementary Figure S26. Denoised, Contrast-enhanced Breast Cancer MIBI Images with Pixel- and Object-based Segmentations by QuPath-StarDist



Top row: *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 1. *Middle:* Pixel-based segmentation analysis by QuPath-StarDist on breast cancer MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath-StarDist on breast cancer MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 2. *Middle:* Pixel-based segmentation analysis by QuPath-StarDist on breast cancer MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath-StarDist on breast cancer MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 3. *Middle:* Pixel-based segmentation analysis by QuPath-StarDist on breast cancer MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath-StarDist applied to breast cancer MIBI Image 3.

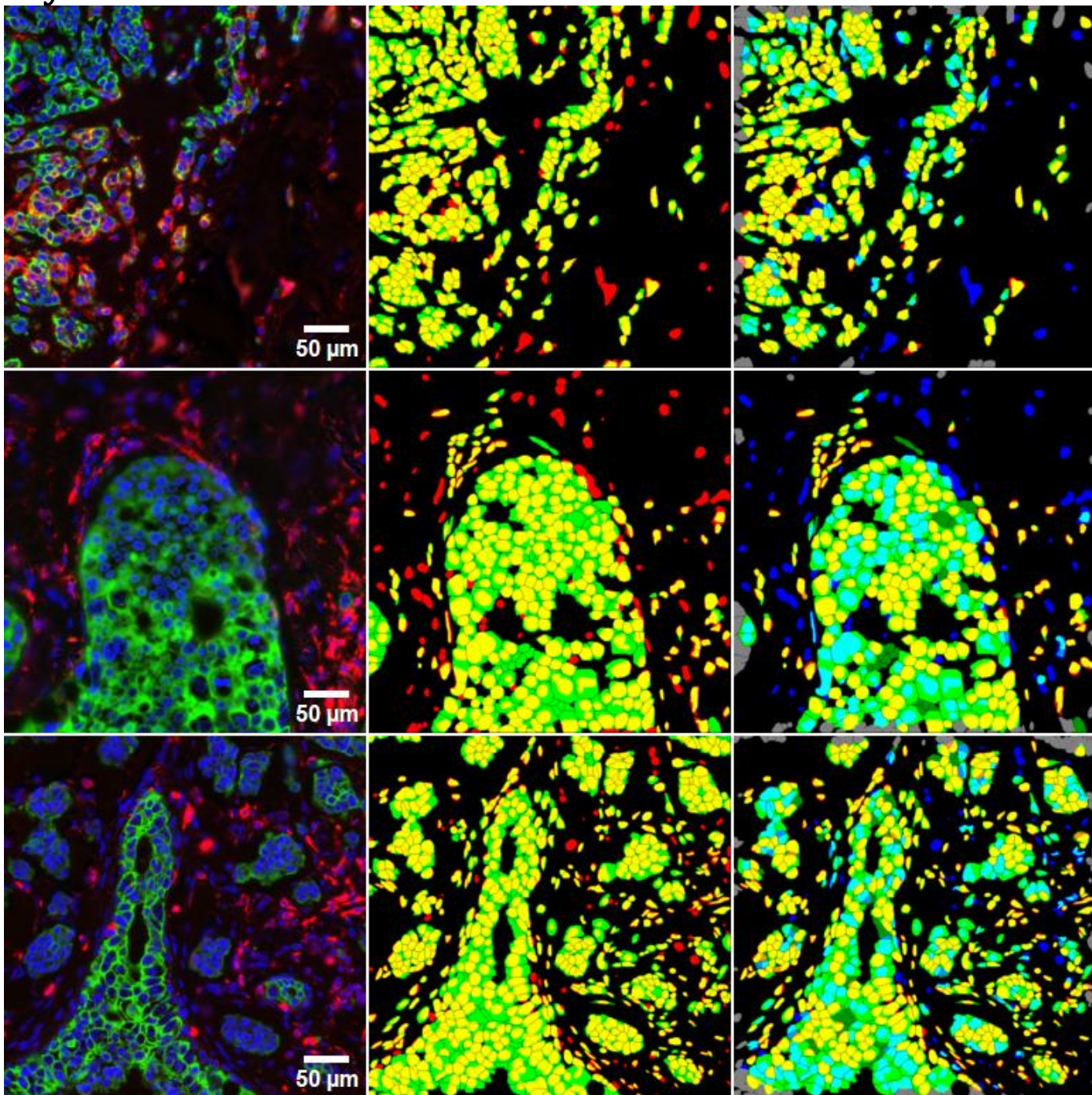
Supplementary Figure S27. Denoised, Contrast-enhanced Breast Cancer MIBI Images with Pixel- and Object-based Segmentations by QuPath-InstanSeg



Top row: *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 1. *Middle:* Pixel-based segmentation analysis by QuPath-InstanSeg on breast cancer MIBI Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath-InstanSeg on breast cancer MIBI Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 2. *Middle:* Pixel-based segmentation analysis by QuPath-InstanSeg on breast cancer MIBI Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath-InstanSeg on breast cancer MIBI Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer MIBI Image 3. *Middle:* Pixel-based segmentation analysis by QuPath-InstanSeg on breast cancer MIBI Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath-InstanSeg applied to breast cancer MIBI Image 3.

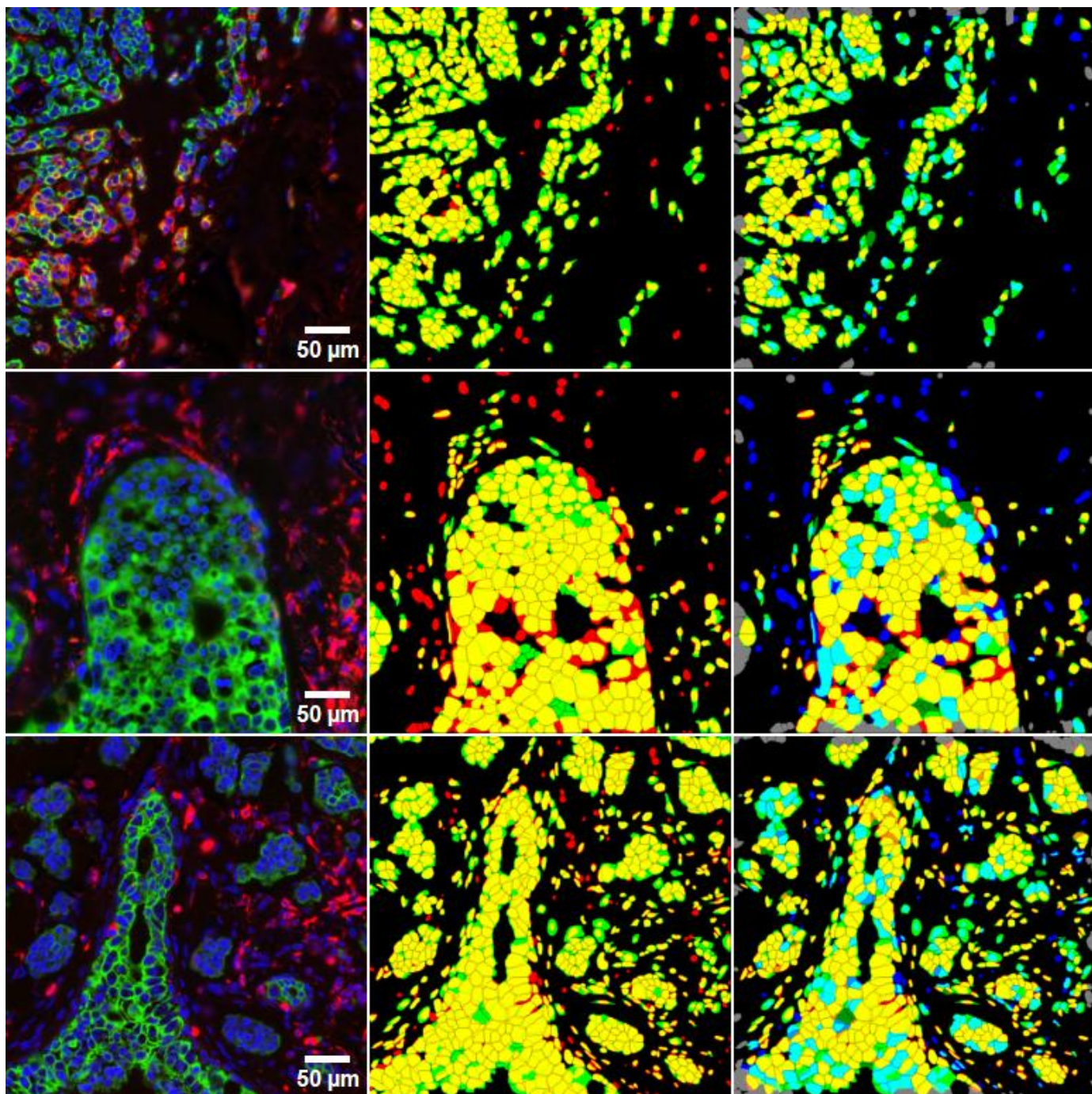
Supplementary Figures for Breast Cancer Lunaphore Images

Supplementary Figure S28. Denoised, Contrast-enhanced Breast Cancer Lunaphore Images with Pixel- and Object-based Segmentations by the Cellpose3-Cyto3 Model



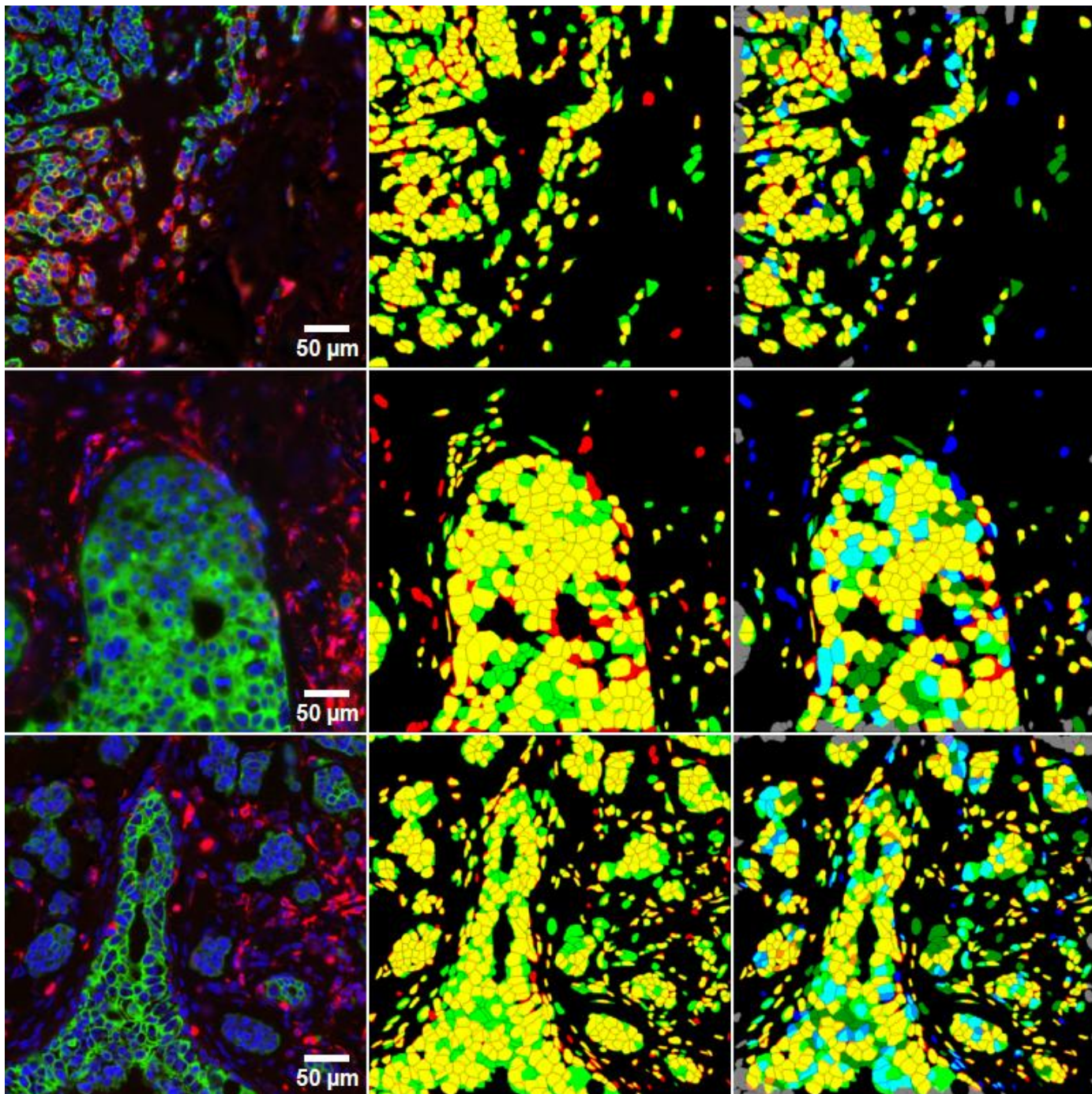
Top row: *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 1. *Middle:* Pixel-based segmentation analysis by Cellpose3-Cyto3 on breast cancer Lunaphore Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose3-Cyto3 on breast cancer Lunaphore Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 2. *Middle:* Pixel-based segmentation analysis by Cellpose3-Cyto3 on breast cancer Lunaphore Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose3-Cyto3 on breast cancer Lunaphore Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 3. *Middle:* Pixel-based segmentation analysis by Cellpose3-Cyto3 on breast cancer Lunaphore Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose3-Cyto3 applied to breast cancer Lunaphore Image 3.

Supplementary Figure S29. Denoised, Contrast-enhanced Breast Cancer Lunaphore Images with Pixel- and Object-based Segmentations by the Cellpose3-Tissue Net Model



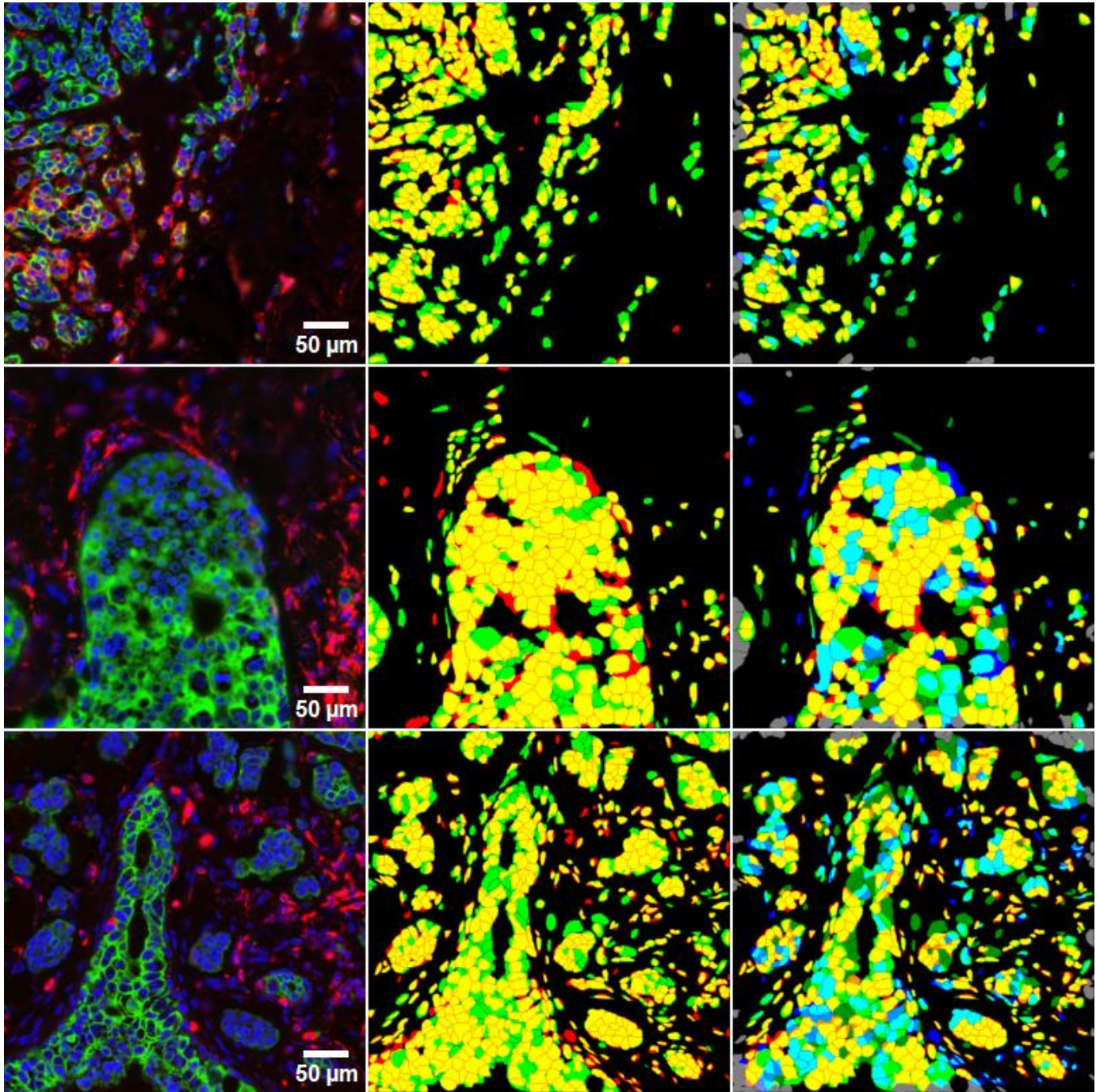
Top row: *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 1. *Middle:* Pixel-based segmentation analysis by Cellpose3-Tissue Net on breast cancer Lunaphore Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose3-Tissue Net on breast cancer Lunaphore Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 2. *Middle:* Pixel-based segmentation analysis by Cellpose3-Tissue Net on breast cancer Lunaphore Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose3-Tissue Net on breast cancer Lunaphore Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 3. *Middle:* Pixel-based segmentation analysis by Cellpose3-Tissue Net on breast cancer Lunaphore Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose3-Tissue Net applied to breast cancer Lunaphore Image 3.

Supplementary Figure S30. Denoised, Contrast-enhanced Breast Cancer Lunaphore Images with Pixel- and Object-based Segmentations by the Cellpose3 Trained Models



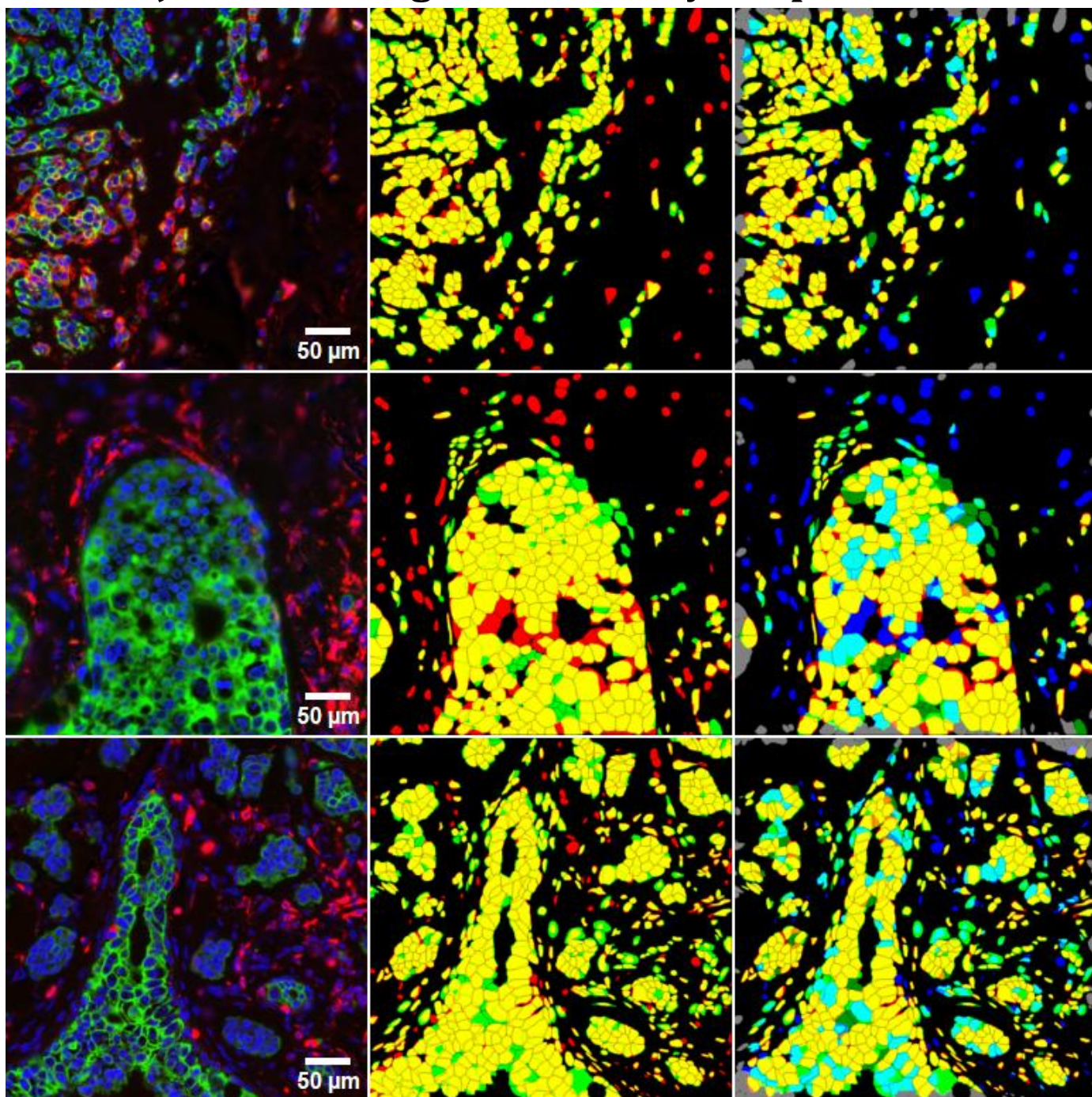
Top row: *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 1. *Middle:* Pixel-based segmentation analysis by Cellpose3 trained models on breast cancer Lunaphore Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3 trained models on breast cancer Lunaphore Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 2. *Middle:* Pixel-based segmentation analysis by Cellpose3 trained models on breast cancer Lunaphore Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose3 trained models on breast cancer Lunaphore Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 3. *Middle:* Pixel-based segmentation analysis by the Cellpose3 trained models on breast cancer Lunaphore Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3 trained models on breast cancer Lunaphore Image 3.

Supplementary Figure S31. Denoised, Contrast-enhanced Breast Cancer Lunaphore Images with Pixel- and Object-based Segmentations by the Cellpose3 Models Trained with Augmented Data



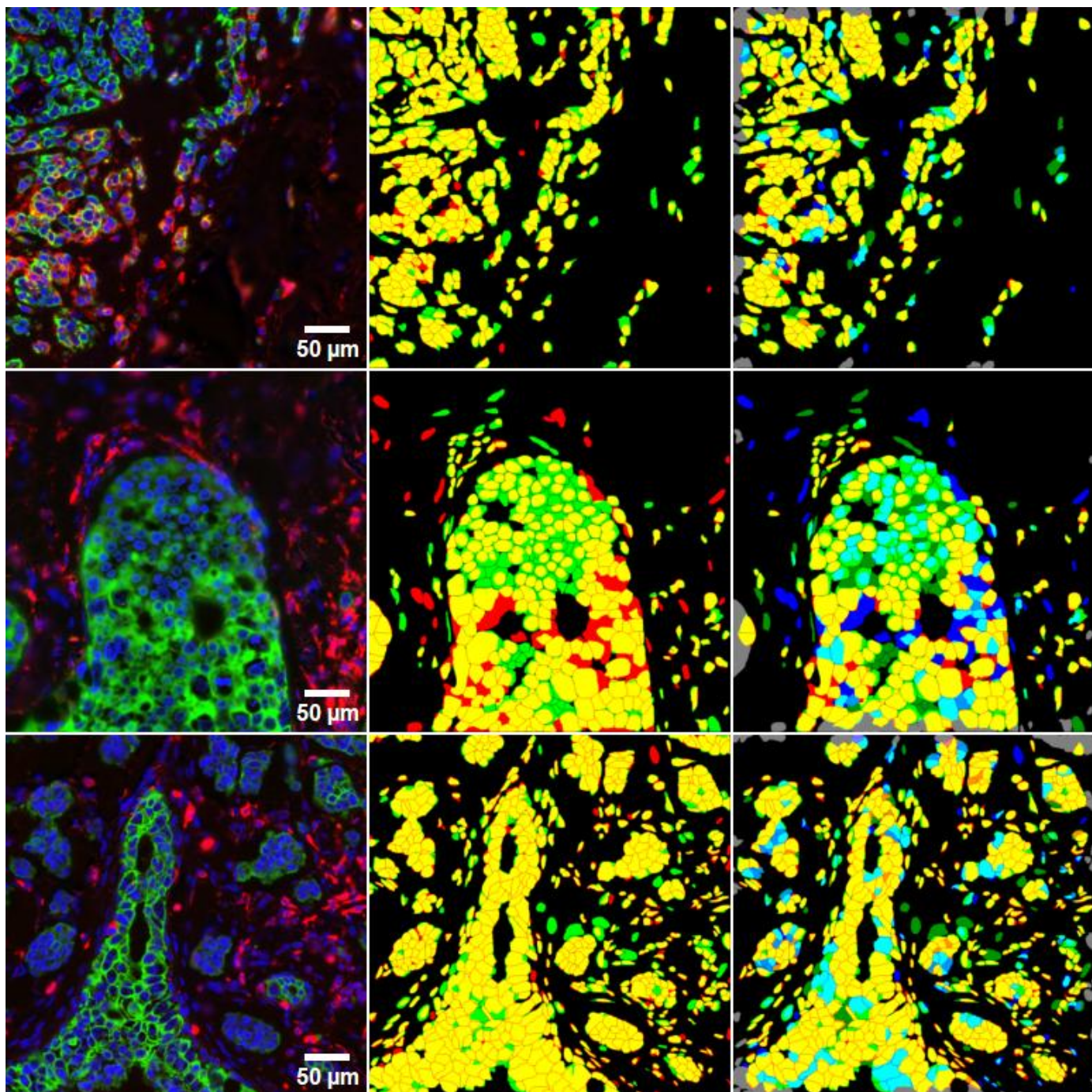
Top row: *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 1. *Middle:* Pixel-based segmentation analysis by Cellpose3 models trained with augmented data on breast cancer Lunaphore Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3 models trained with augmented data on breast cancer Lunaphore Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 2. *Middle:* Pixel-based segmentation analysis by Cellpose3 models trained with augmented data on breast cancer Lunaphore Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose3 models trained with augmented data on breast cancer Lunaphore Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 3. *Middle:* Pixel-based segmentation analysis by the Cellpose3 models trained with augmented data on breast cancer Lunaphore Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by the Cellpose3 models trained with augmented data on breast cancer Lunaphore Image 3.

Supplementary Figure S32. Denoised, Contrast-enhanced Breast Cancer Lunaphore Images with Pixel- and Object-based Segmentations by Cellpose4-SAM



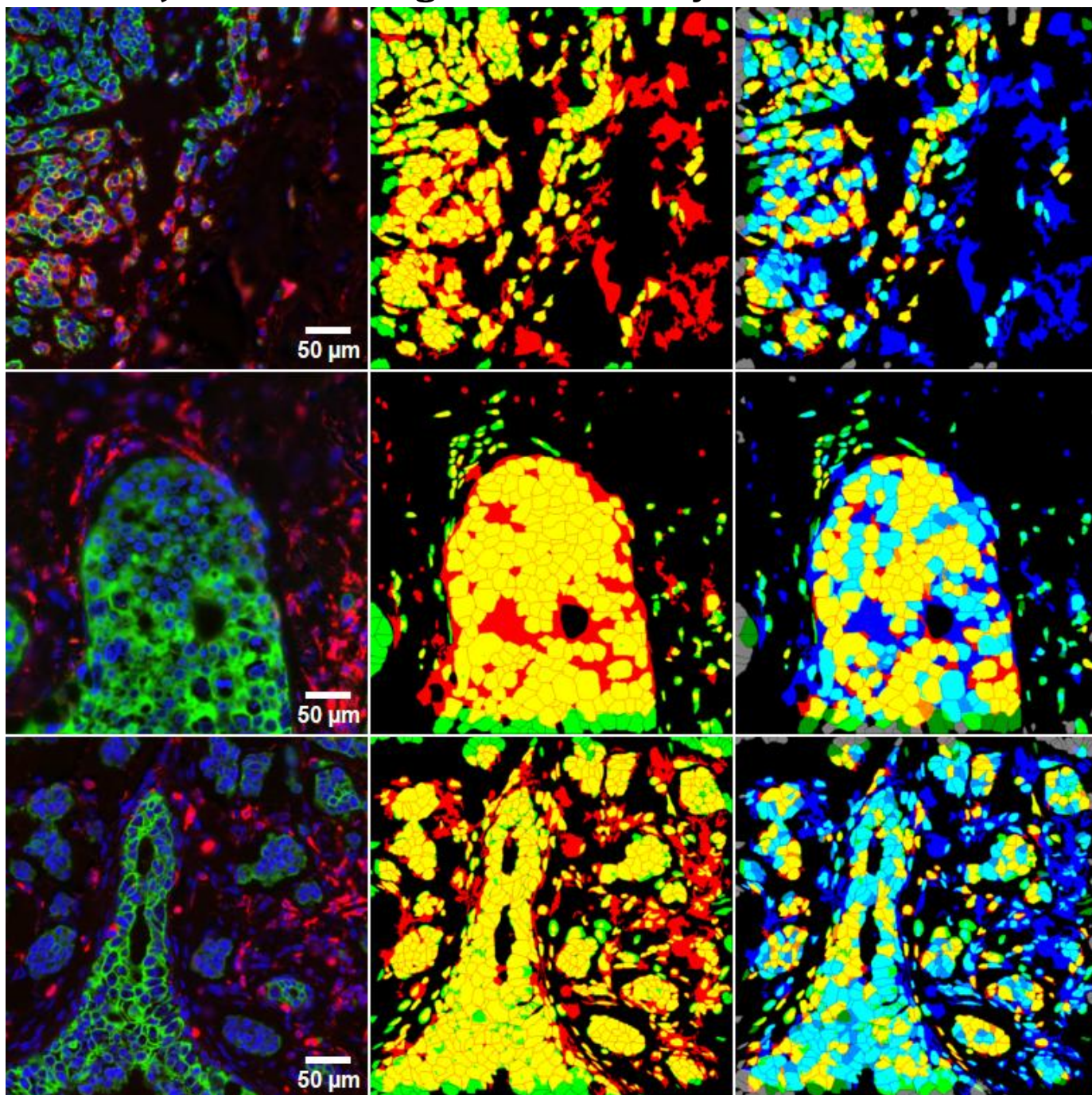
Top row: *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 1. *Middle:* Pixel-based segmentation analysis by Cellpose4-SAM on breast cancer Lunaphore Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose4-SAM on breast cancer Lunaphore Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 2. *Middle:* Pixel-based segmentation analysis by Cellpose4-SAM on breast cancer Lunaphore Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose4-SAM on breast cancer Lunaphore Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 3. *Middle:* Pixel-based segmentation analysis by Cellpose4-SAM on breast cancer Lunaphore Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose4-SAM applied to breast cancer Lunaphore Image 3.

Supplementary Figure S33. Denoised, Contrast-enhanced Breast Cancer Lunaphore Images with Pixel- and Object-based Segmentations by Cellpose4-SAM Trained Models



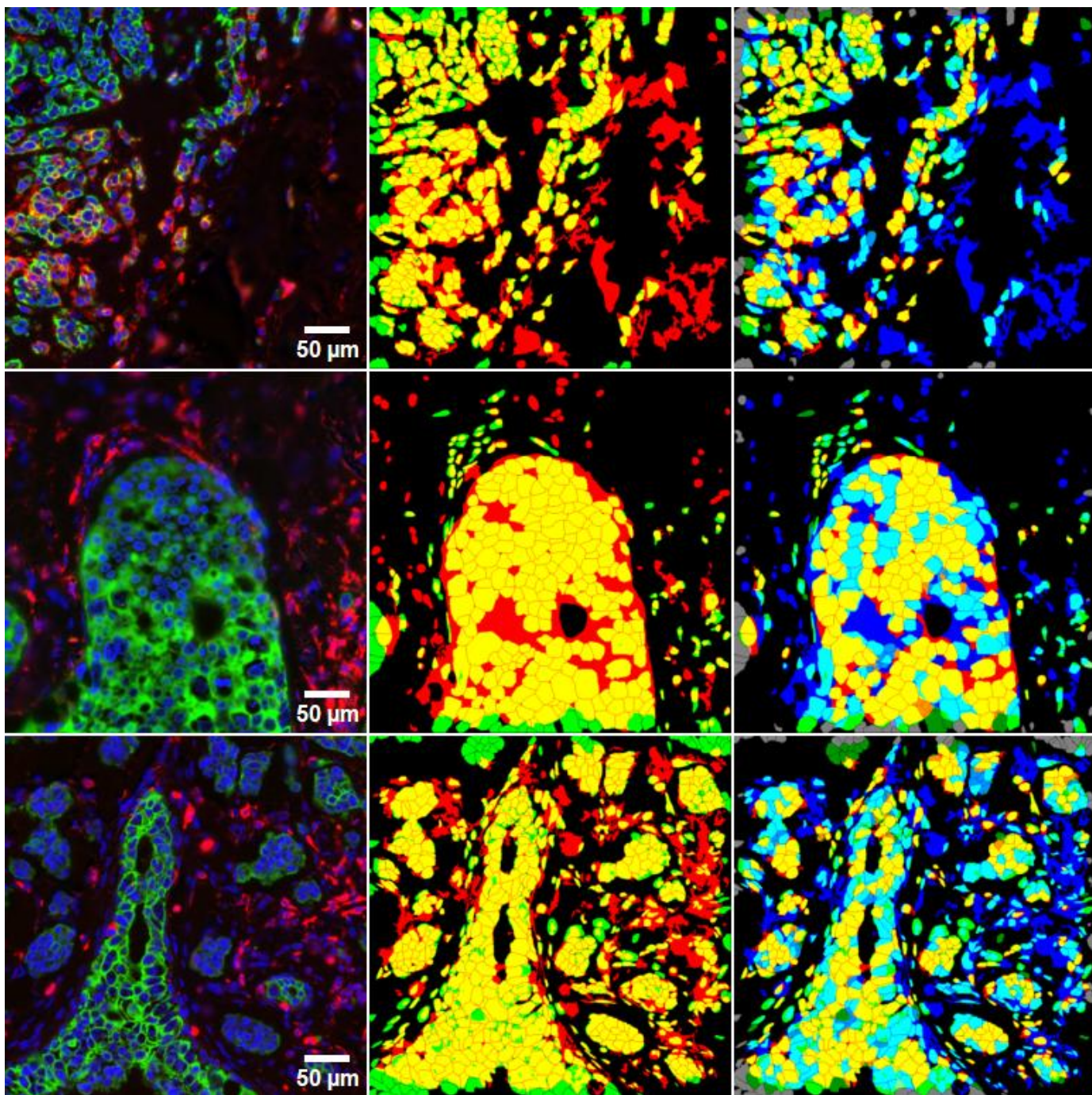
Top row: *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 1. *Middle:* Pixel-based segmentation analysis by Cellpose4-SAM trained models on breast cancer Lunaphore Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose4-SAM trained models on breast cancer Lunaphore Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 2. *Middle:* Pixel-based segmentation analysis by Cellpose4-SAM trained models on breast cancer Lunaphore Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose4-SAM trained models on breast cancer Lunaphore Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 3. *Middle:* Pixel-based segmentation analysis by Cellpose4-SAM trained models on breast cancer Lunaphore Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by Cellpose4-SAM trained models applied to breast cancer Lunaphore Image 3.

Supplementary Figure S34. Denoised, Contrast-enhanced Breast Cancer Lunaphore Images with Pixel- and Object-based Segmentations by CellProfiler



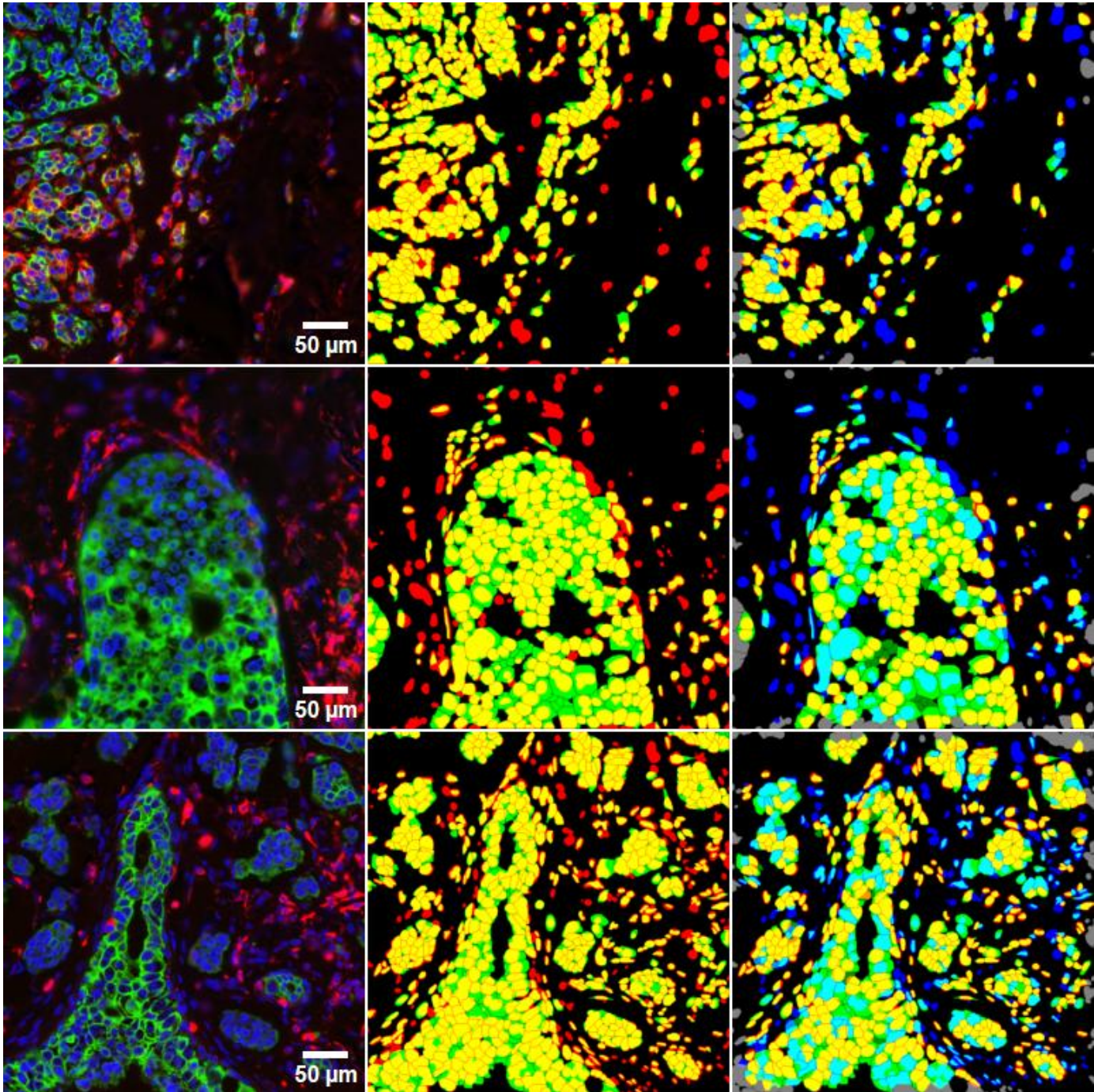
Top row: *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 1. *Middle:* Pixel-based segmentation analysis by CellProfiler on breast cancer Lunaphore Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by CellProfiler on breast cancer Lunaphore Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 2. *Middle:* Pixel-based segmentation analysis by CellProfiler on breast cancer Lunaphore Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by CellProfiler on breast cancer Lunaphore Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 3. *Middle:* Pixel-based segmentation analysis by CellProfiler on breast cancer Lunaphore Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by CellProfiler applied to breast cancer Lunaphore Image 3.

Supplementary Figure S35. Denoised, Contrast-enhanced Breast Cancer Lunaphore Images with Pixel- and Object-based Segmentations by CellProfiler-StarDist



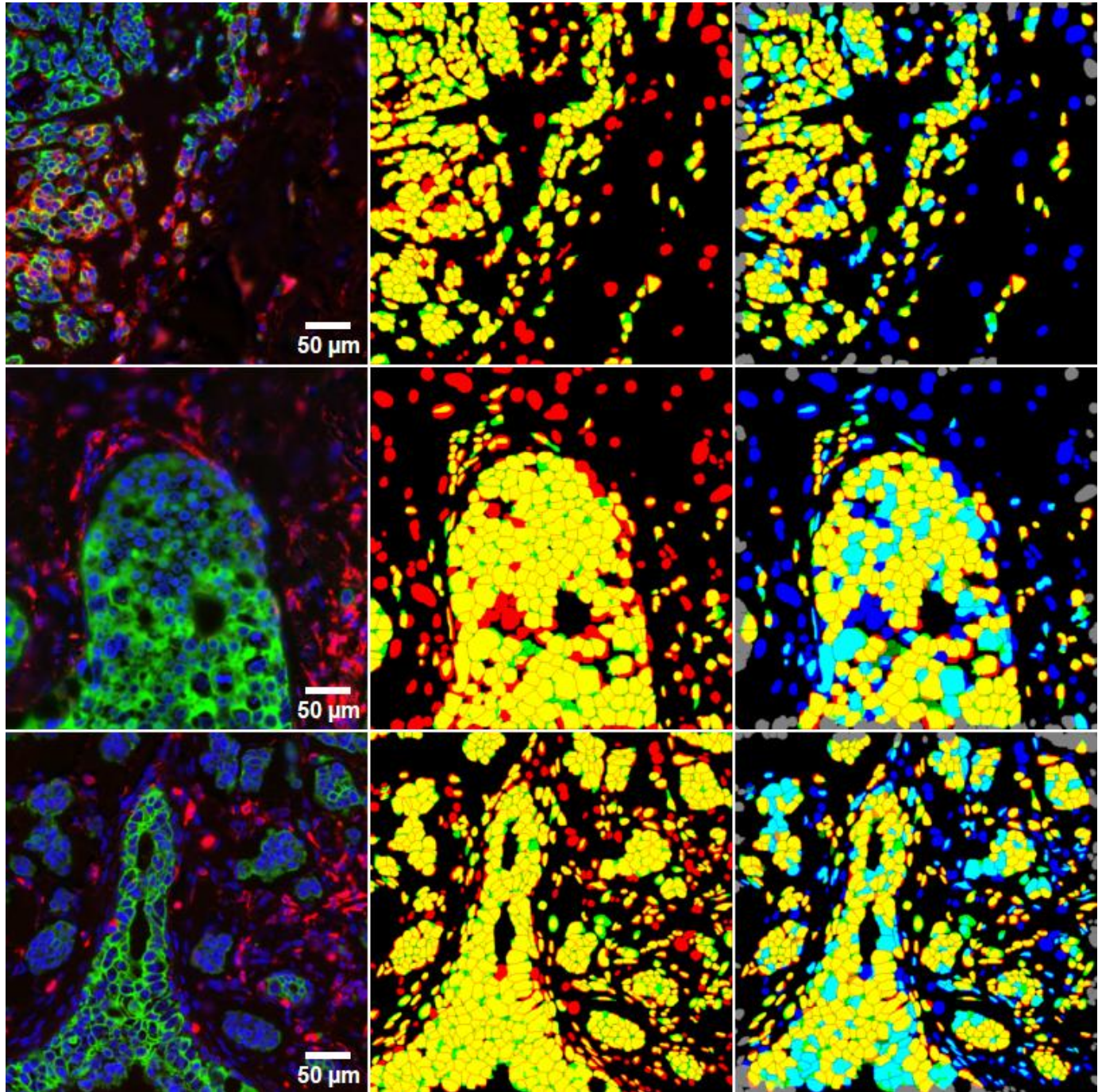
Top row: *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 1. *Middle:* Pixel-based segmentation analysis by CellProfiler-StarDist on breast cancer Lunaphore Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by CellProfiler-StarDist on breast cancer Lunaphore Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 2. *Middle:* Pixel-based segmentation analysis by CellProfiler-StarDist on breast cancer Lunaphore Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by CellProfiler-StarDist on breast cancer Lunaphore Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 3. *Middle:* Pixel-based segmentation analysis by CellProfiler-StarDist on breast cancer Lunaphore Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by CellProfiler-StarDist applied to breast cancer Lunaphore Image 3.

Supplementary Figure S36. Denoised, Contrast-enhanced Breast Cancer Lunaphore Images with Pixel- and Object-based Segmentations by Mesmer



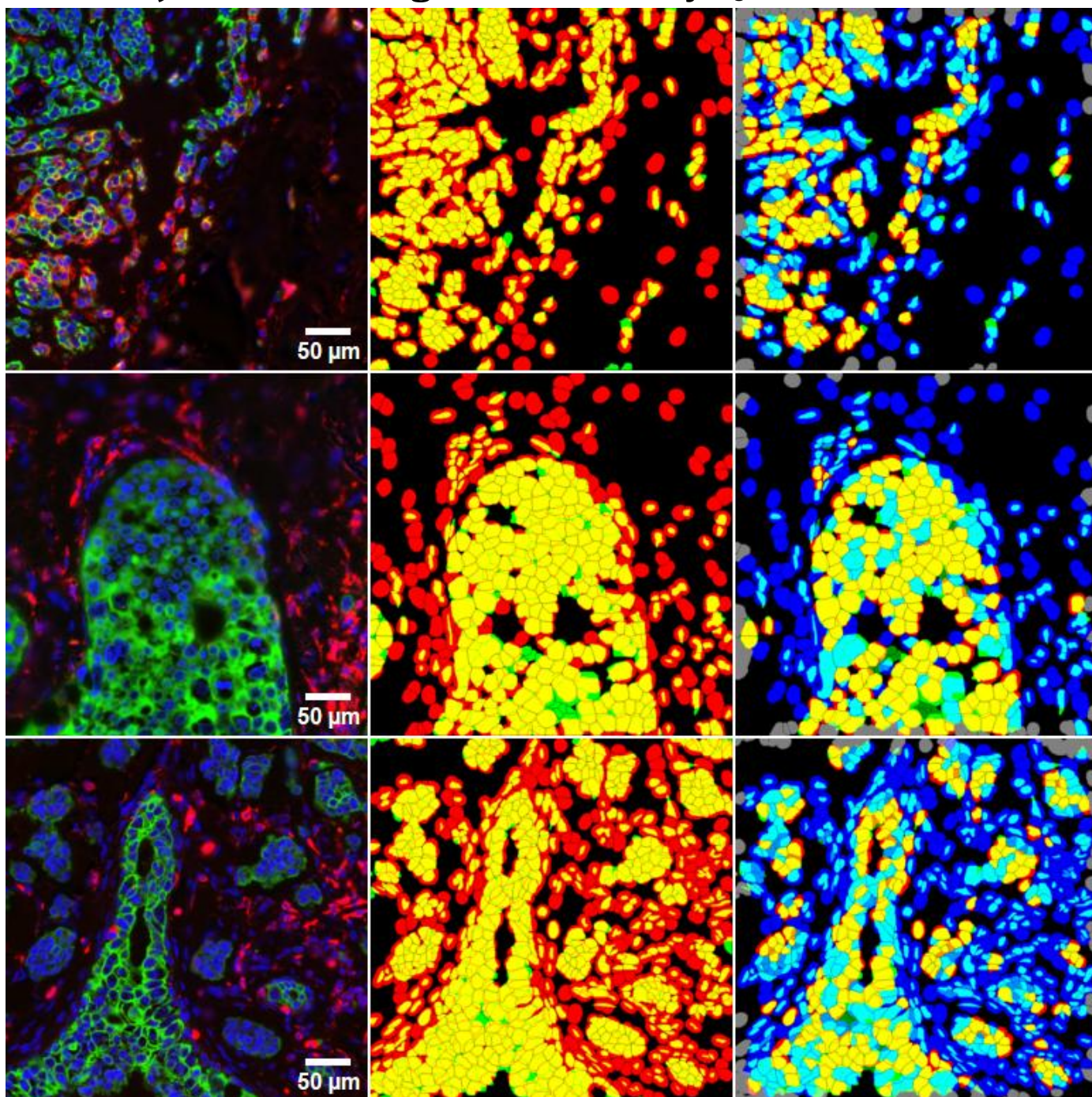
Top row: *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 1. *Middle:* Pixel-based segmentation analysis by Mesmer on breast cancer Lunaphore Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by Mesmer on breast cancer Lunaphore Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 2. *Middle:* Pixel-based segmentation analysis by Mesmer on breast cancer Lunaphore Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by Mesmer on breast cancer Lunaphore Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 3. *Middle:* Pixel-based segmentation analysis by Mesmer on breast cancer Lunaphore Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by Mesmer applied to breast cancer Lunaphore Image 3.

Supplementary Figure S37. Denoised, Contrast-enhanced Breast Cancer Lunaphore Images with Pixel- and Object-based Segmentations by napari-CellSAM



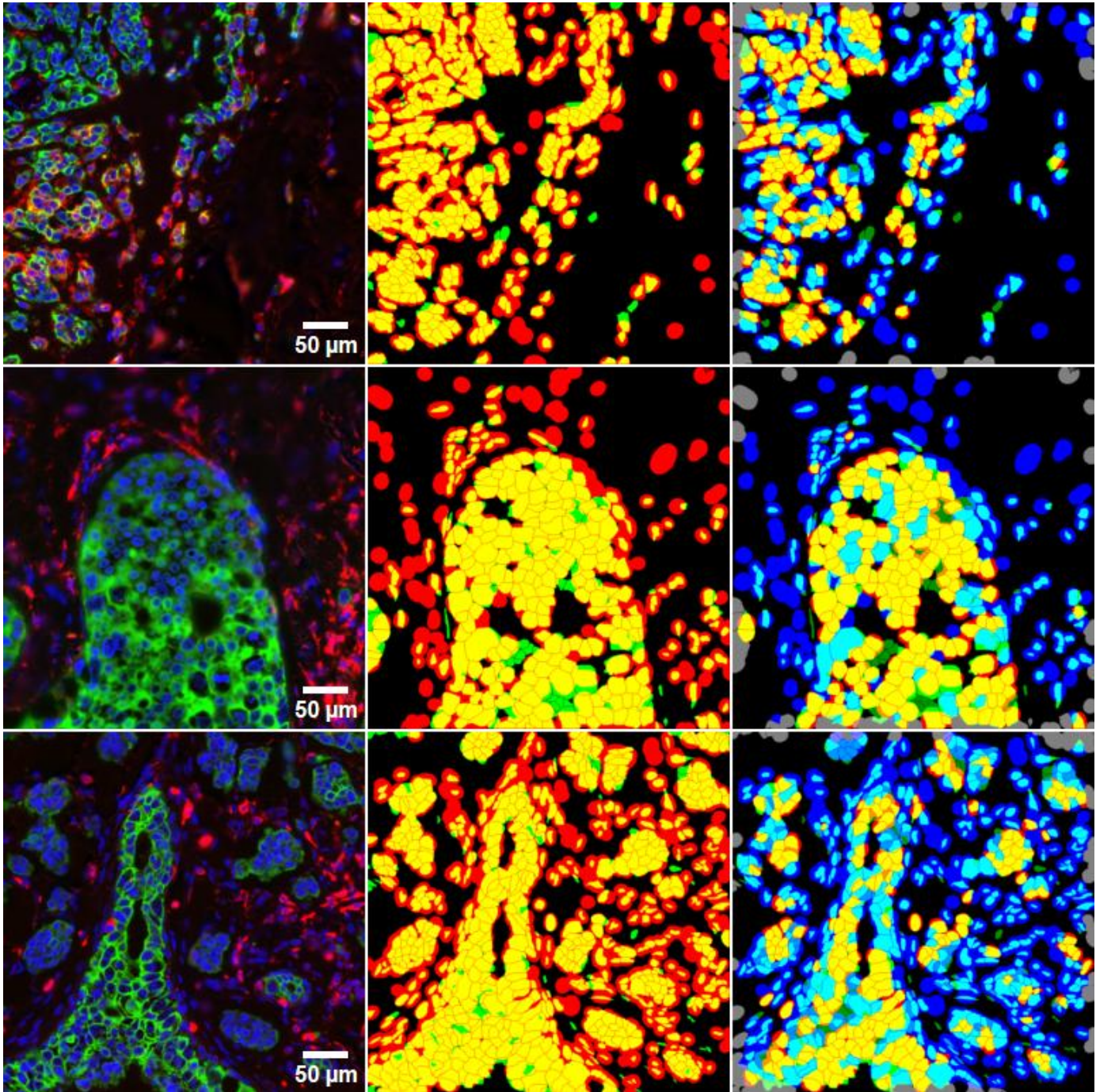
Top row: *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 1. *Middle:* Pixel-based segmentation analysis by napari-CellSAM on breast cancer Lunaphore Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by napari-CellSAM on breast cancer Lunaphore Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 2. *Middle:* Pixel-based segmentation analysis by napari-CellSAM on breast cancer Lunaphore Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by napari-CellSAM on breast cancer Lunaphore Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 3. *Middle:* Pixel-based segmentation analysis by napari-CellSAM on breast cancer Lunaphore Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by napari-CellSAM applied to breast cancer Lunaphore Image 3.

Supplementary Figure S38. Denoised, Contrast-enhanced Breast Cancer Lunaphore Images with Pixel- and Object-based Segmentations by QuPath



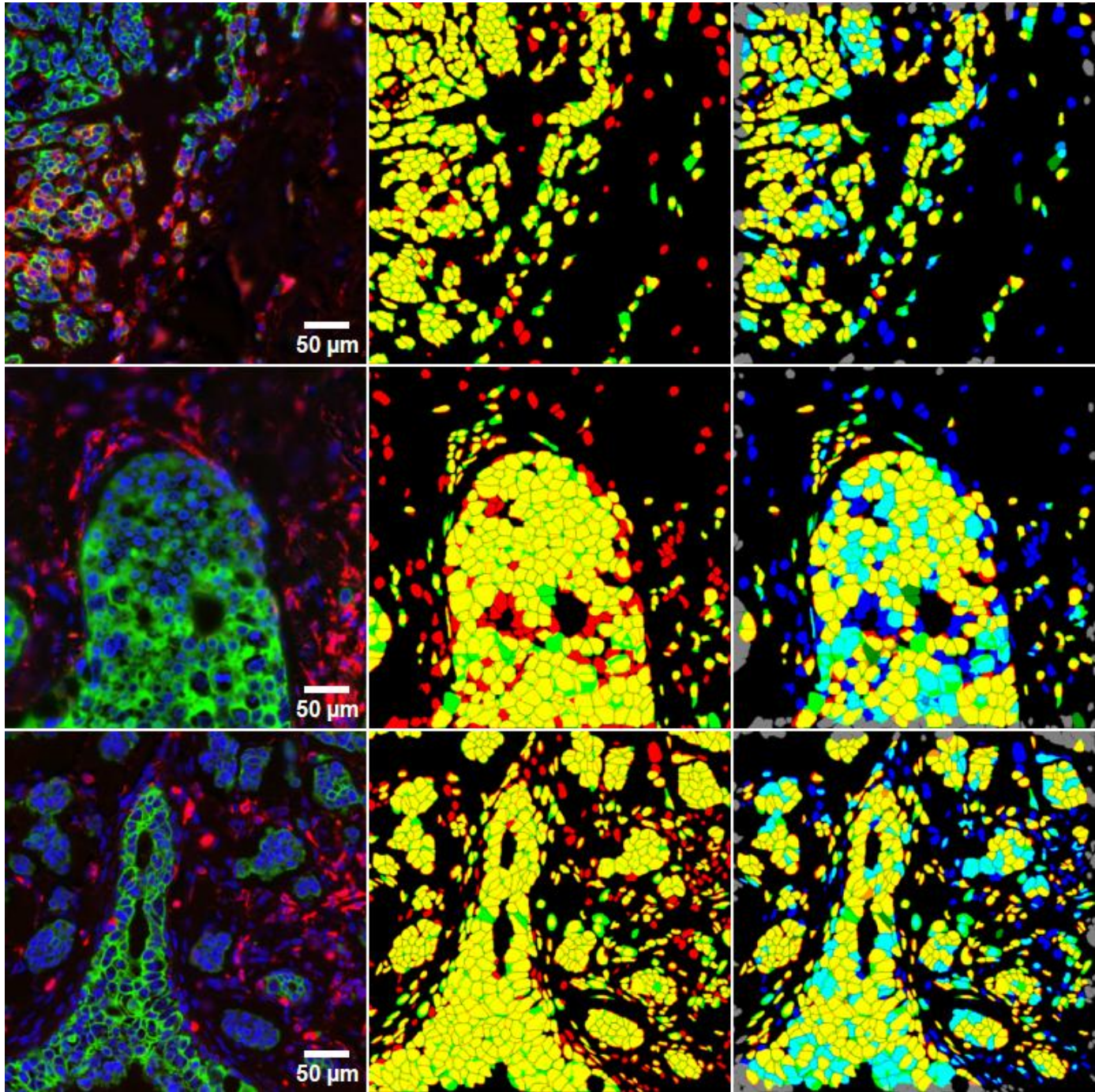
Top row: *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 1. *Middle:* Pixel-based segmentation analysis by QuPath on breast cancer Lunaphore Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath on breast cancer Lunaphore Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 2. *Middle:* Pixel-based segmentation analysis by QuPath on breast cancer Lunaphore Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath on breast cancer Lunaphore Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 3. *Middle:* Pixel-based segmentation analysis by QuPath on breast cancer Lunaphore Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath applied to breast cancer Lunaphore Image 3.

Supplementary Figure S39. Denoised, Contrast-enhanced Breast Cancer Lunaphore Images with Pixel- and Object-based Segmentations by QuPath-StarDist



Top row: *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 1. *Middle:* Pixel-based segmentation analysis by QuPath-StarDist on breast cancer Lunaphore Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath-StarDist on breast cancer Lunaphore Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 2. *Middle:* Pixel-based segmentation analysis by QuPath-StarDist on breast cancer Lunaphore Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath-StarDist on breast cancer Lunaphore Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 3. *Middle:* Pixel-based segmentation analysis by QuPath-StarDist on breast cancer Lunaphore Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath-StarDist applied to breast cancer Lunaphore Image 3.

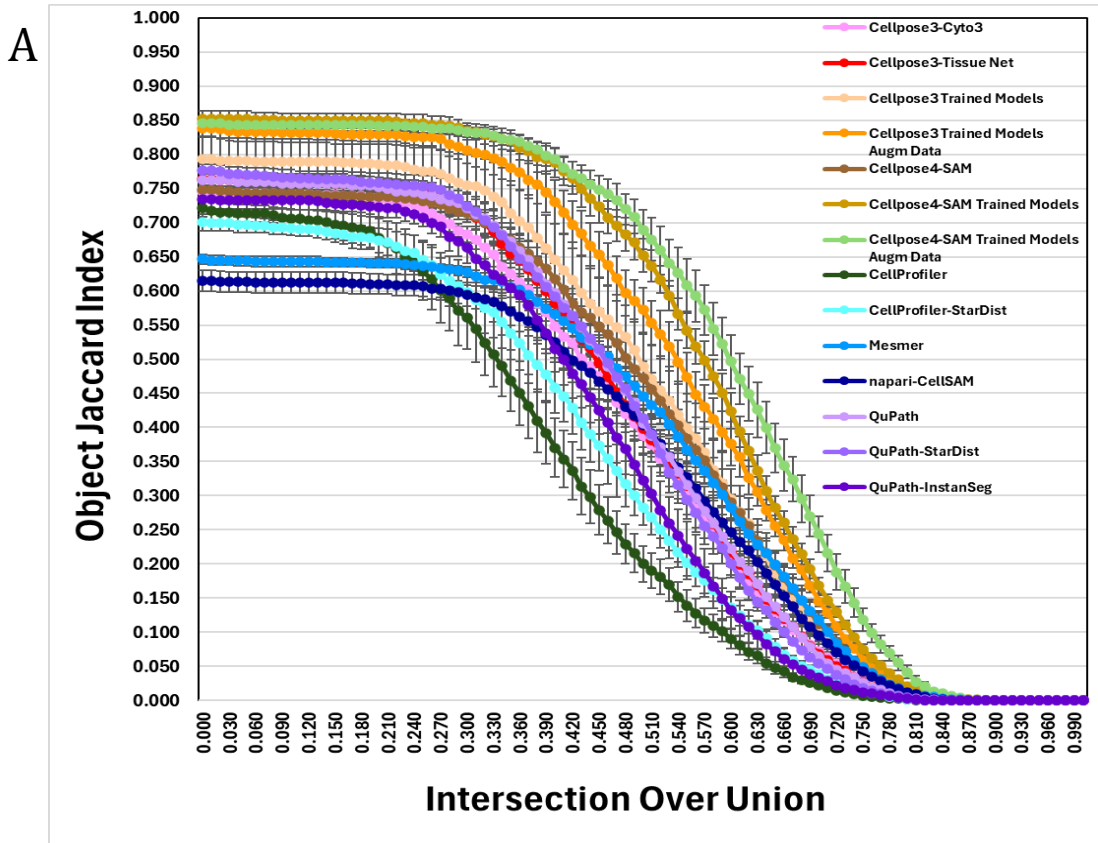
Supplementary Figure S40. Denoised, Contrast-enhanced Breast Cancer Lunaphore Images with Pixel- and Object-based Segmentations by QuPath-InstanSeg



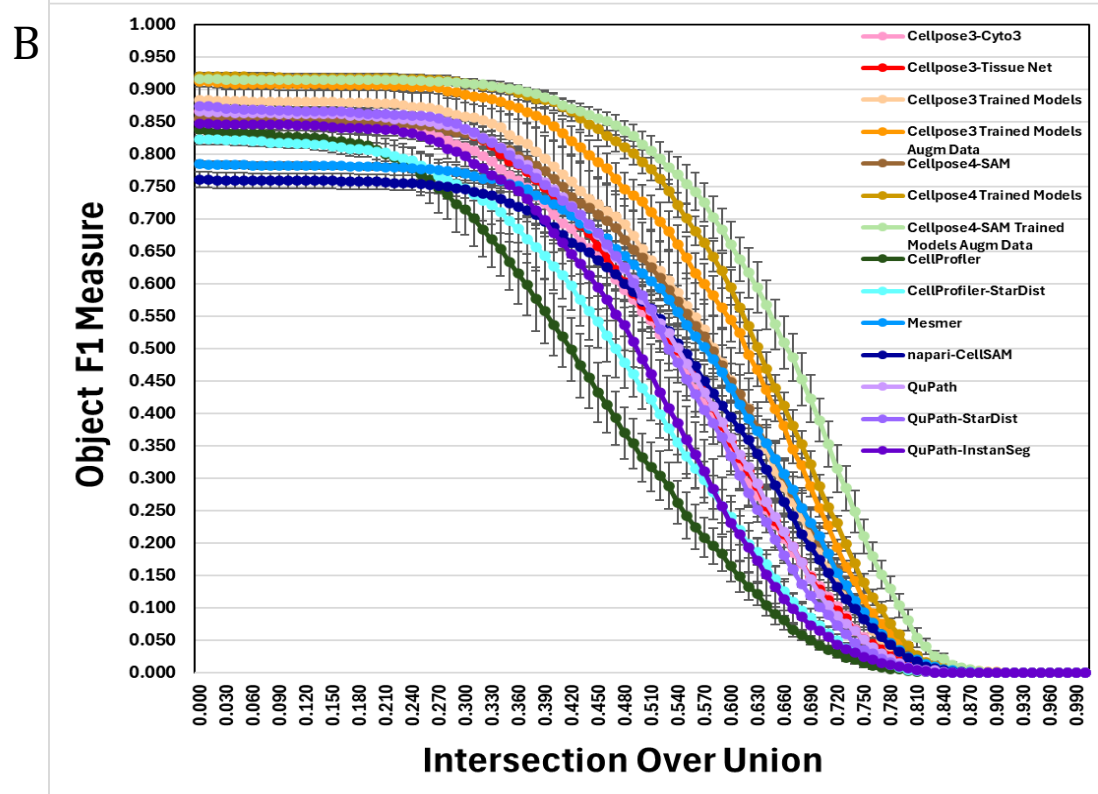
Top row: *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 1. *Middle:* Pixel-based segmentation analysis by QuPath-InstanSeg on breast cancer Lunaphore Image 1. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath-InstanSeg on breast cancer Lunaphore Image 1. **Middle Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 2. *Middle:* Pixel-based segmentation analysis by QuPath-InstanSeg on breast cancer Lunaphore Image 2. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath-InstanSeg on breast cancer Lunaphore Image 2. **Bottom Row:** *Left:* Denoised, contrast-enhanced breast cancer Lunaphore Image 3. *Middle:* Pixel-based segmentation analysis by QuPath-InstanSeg on breast cancer Lunaphore Image 3. *Right:* Object-based segmentation analysis (IoU=0.5) by QuPath-InstanSeg applied to breast cancer Lunaphore Image 3.

Supplementary Figure S41. The Cellpose4-SAM Models Trained with Augmented Data Provide the Highest Segmentation Overlap and Accuracy in melanoma MIBI Images. Open-source software segmentation analysis on melanoma MIBI images. The Cellpose4-SAM models trained with augmented data gave the highest segmentation overlap and accuracy, followed by the Cellpose4 trained models and the Cellpose3 models trained with augmented data. The next grouping had several software performing within each other's margin of error: native Cellpose4-SAM, Mesmer, napari-CellSAM, QuPath, QuPath-StarDist, Cellpose3 models trained without augmented data, and Cellpose3-TissueNet and -Cyto3. The final three software with the lowest segmentation overlap and accuracy were QuPath-InstanSeg, CellProfiler-StarDist, and CellProfiler.

Plot of object Jaccard index versus intersection over union (IoU) metric.

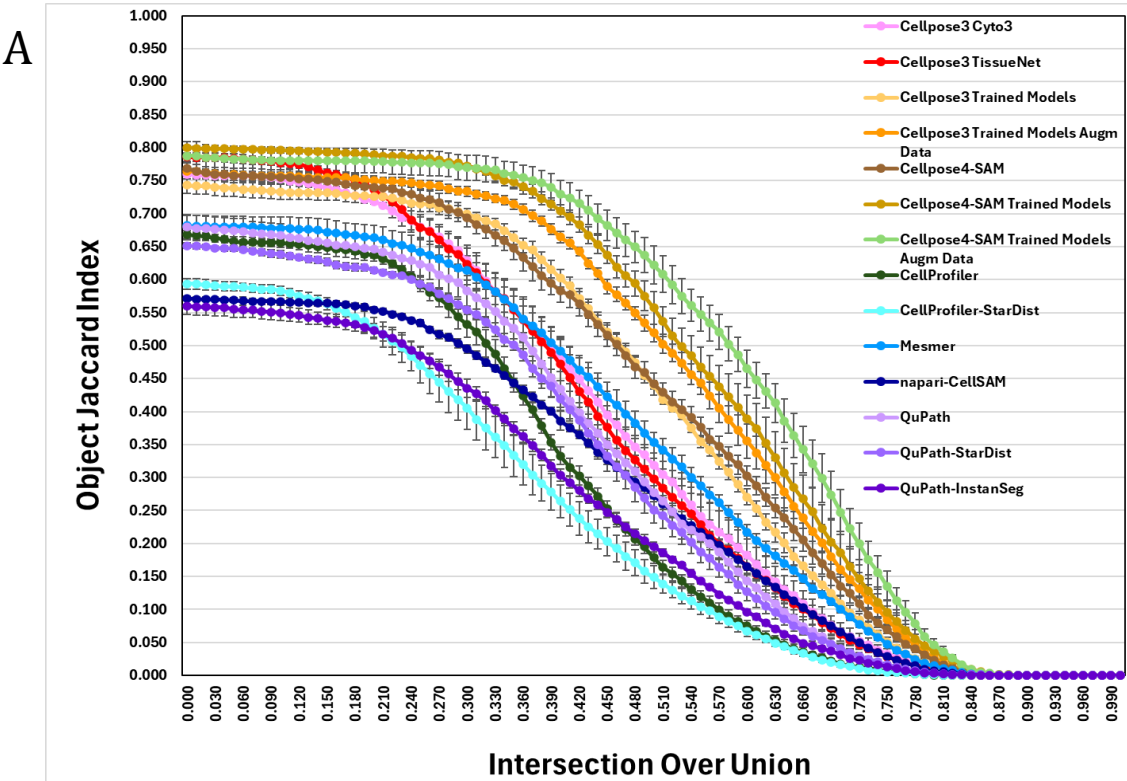


Plot of object F1 measure versus intersection over union (IoU) metric.

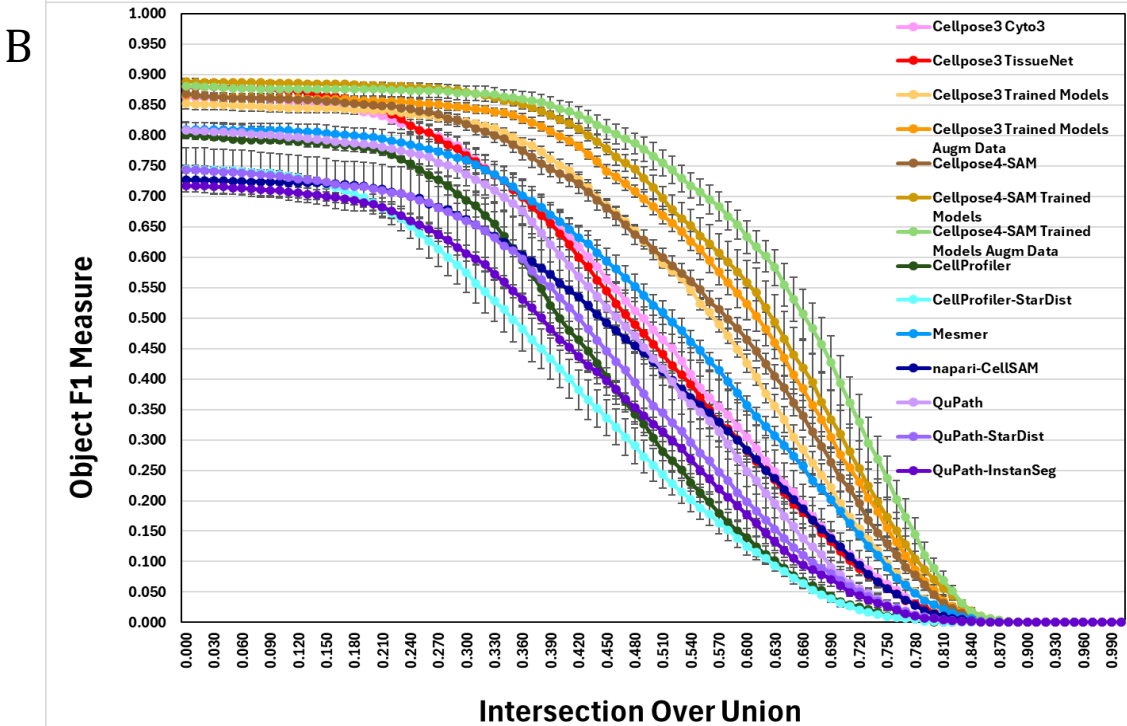


Supplementary Figure S42. The Cellpose4-SAM Models Trained with Augmented Data Provide the Highest Segmentation Overlap and Accuracy in Breast Cancer MIBI Images. Open-source software segmentation analysis on breast cancer MIBI images. The Cellpose4-SAM models trained with augmented data gave the highest segmentation overlap and accuracy, with the Cellpose3 trained models and the native Cellpose4-SAM model both performing similarly. The next grouping had several software performing within each other's margin of error: Mesmer, napari-CellSAM, QuPath, QuPath-StarDist, and Cellpose3-TissueNet and -Cyto3. The final three software with the lowest segmentation overlap and accuracy were QuPath-InstanSeg, CellProfiler-StarDist, and CellProfiler.

Plot of object Jaccard index versus intersection over union (IoU) metric.



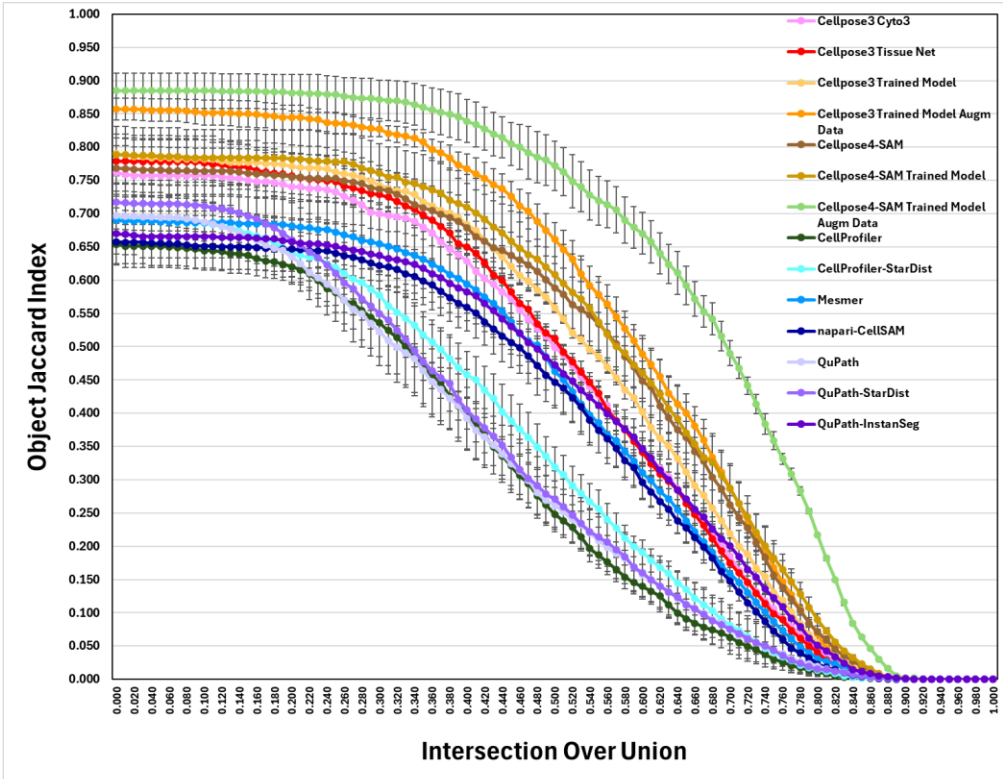
Plot of object F1 measure versus intersection over union (IoU) metric.



Supplementary Figure S43. The Cellpose4-SAM Models Trained with Augmented Data Provide the Highest Segmentation Overlap and Accuracy in Denoised, Contrast-enhanced Breast Cancer Lunaphore Images. Open-source software segmentation analysis on Lunaphore breast cancer images. The Cellpose4-SAM models trained with and without augmented data gave the highest segmentation overlap and accuracy, followed by the Cellpose3 models trained with augmented data. The middle group of software included the Cellpose4 trained models, native Cellpose4-SAM, Cellpose 3 trained models, Cellpose3-TissueNet, Cellpose3-Cyto3, Mesmer, QuPath-InstanSeg, and napari-CellSAM. The lowest grouping of software included CellProfiler-StarDist, QuPath-StarDist, QuPath, and CellProfiler.

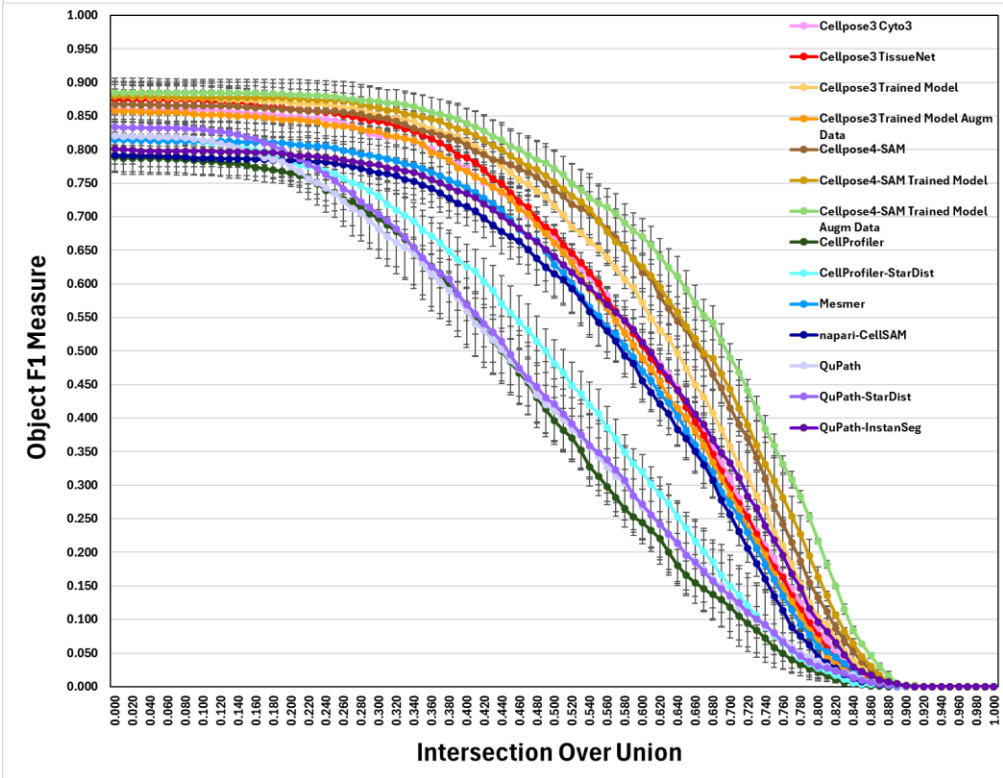
A

Plot of object Jaccard index versus intersection over union (IoU) metric.

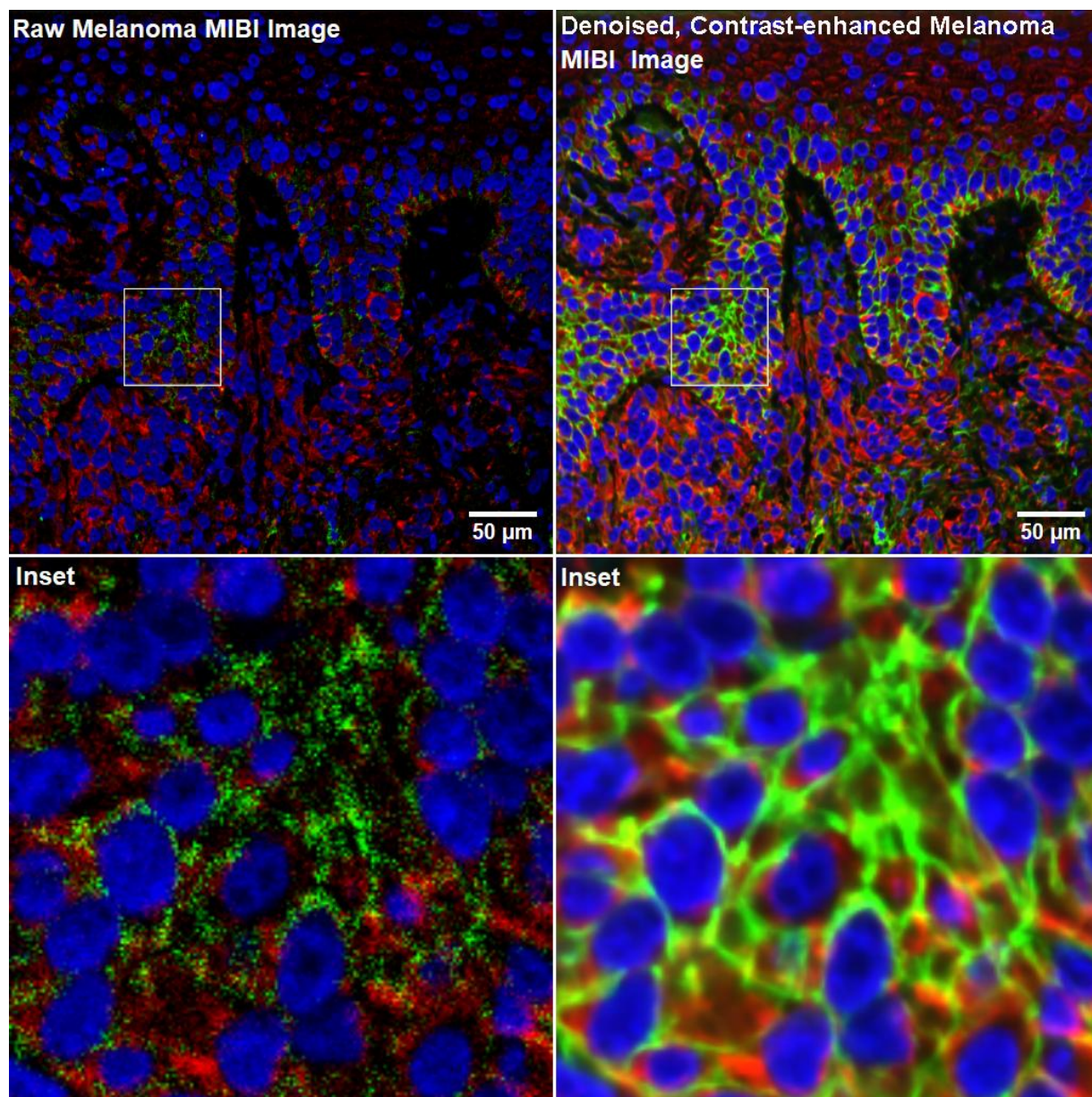


B

Plot of object F1 measure versus intersection over union (IoU) metric.

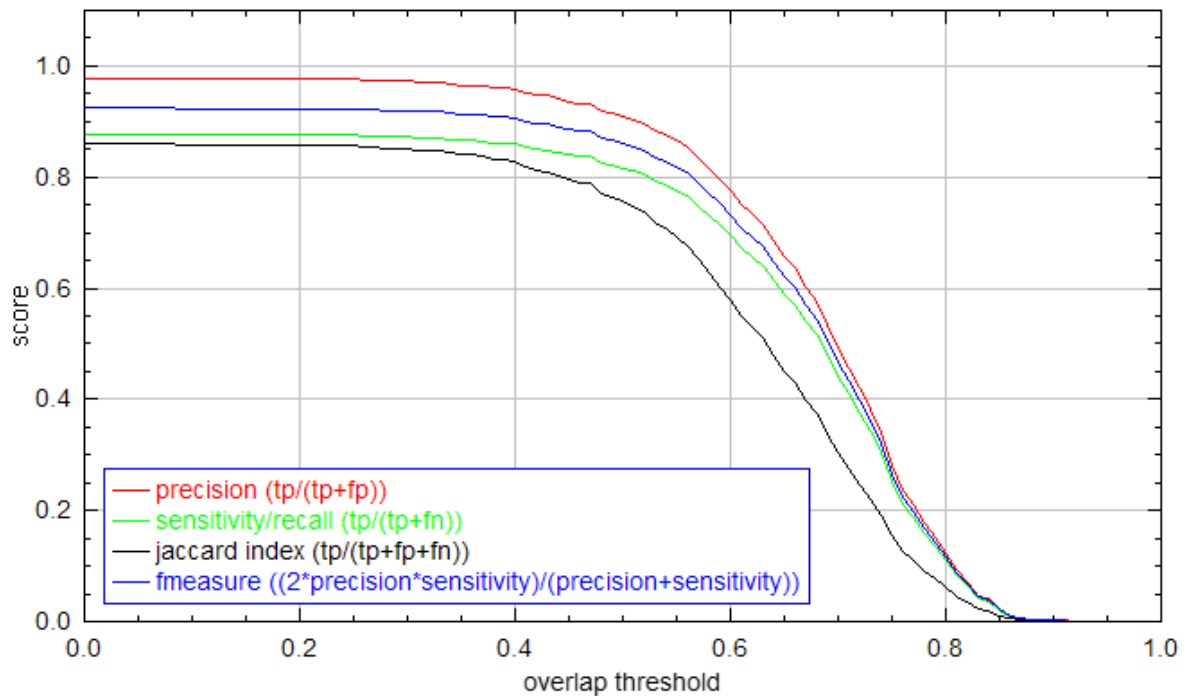


Supplementary Figure S44. Denoising with the Noise2Noise FIJI plugin¹ removed pixelation and smoothed a representative melanoma MIBI image.



¹Mannam et al. Real-time image denoising of mixed Poisson-Gaussian noise in fluorescence microscopy images using ImageJ. *Optica* 2022; 9 (4): 335-345.

Supplementary Figure S45. Example plots of object precision, recall, Jaccard index, and F1 measure across all IoU thresholds, ranging from 0.0 – 1.0 in increments of 0.01, generated by the FIJI Mask Instant Comparator plugin² for every individual image.



²<https://github.com/MultimodalImagingCenter/MiC>

Supplementary Figure 46. Example of pixel-based analysis of cell instance segmentation by the Mask Image Comparator plugin in FIJI. Red pixels indicate false positive detection, green pixels indicate false negative detection, and yellow pixels indicate codetection of GT: test object signals. Black pixels represent true negative background signal.

