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A Robust Deep Learning Approach for Precisely Segmenting Cells in Multiplex Tissue Images

Daniel E. Winkowski, Ph.D., Jenifer Caldara, Brit Boehmer, Ph.D., T. Regan Baird, Ph.D.

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ABSTRACT

Multiplex tissue images are becoming pivotal in tissue pathology because they provide positional location and multidimensional phenotyping of every cell. However, given that tissues contain a heterogenous mixture of unique cellular morphologies and densities, it has been difficult to automatically and accurately define each cell's outer boundary for proper analysis. Cell Segmentation routines that are based solely on nuclei are commonplace, but insufficient, in precisely defining cell edges.

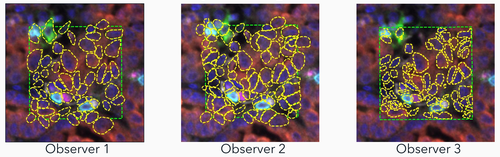
In this study, we have used Visiopharm's image analysis platform to explore 3 different approaches to segment cells in samples stained with varying multiplexed fluorescent assays. Visiopharm offers a complete toolbox for design and customizing your own algorithm but also offers a general nuclei detection algorithm which uses Deep Learning AI to segment nuclei of most morphologies. We then adapted this algorithm, using Visiopharm's authoring tools, to combine its nuclear AI classifier with signals from biomarkers in the multiplex panels to expand the segmentation beyond the nuclei, to the outer cell periphery. The performance of these approaches was then quantified by comparing with manual annotations as a ground truth.

METHODS

Ground truth annotations were prepared by three human observers on images from two different instrument manufacturers; Akoya Vectra Polaris 8-plex lung cancer and Fluidigm Hyperion 13-plex Spleen. The AI's performance was assessed against the ground truth annotations using Precision (a ratio of true and false positives), Sensitivity (a ratio of true positives and false negatives), and DICE Score (a ratio of all true/false, positive/negatives).

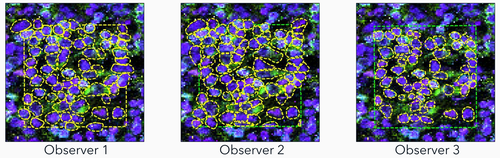
GROUND TRUTH ANNOTATIONS

Akoya Vectra Polaris 8-plex Lung Cancer



Observer 1 | Observer 2 | Observer 3

Fluidigm Hyperion 13-plex Spleen



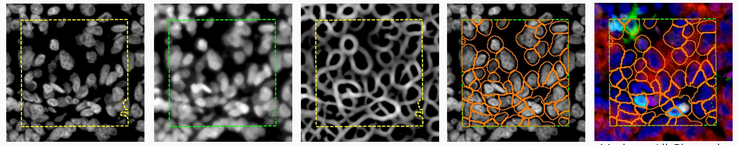
Observer 1 | Observer 2 | Observer 3

Manual annotations were prepared by three unique human observers on images from two different instrument manufacturers. Human observers utilized both nuclear and biomarker signals to aid during annotation placement. Note the variability across observers.

AI-BASED SEGMENTATION

Traditional Image Analysis (Trad)

Built-in filtering methods were used to enhance objects of interest and suppress noise in the image. Such methods are morphometry-based, not intensity-based. Parameter optimization can be time-consuming.

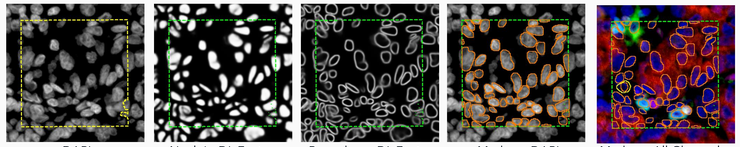


DAPI | DAPI – median filter | Edge Finding filter | Markup+DAPI | Markup+All Channels

Advantages: Nuclei are identified based on shape, not staining intensity
Disadvantages: Algorithm development can be time consuming, Performance can decline with diverse nuclear morphologies (big and small nuclei) in the sample

Ready-To-Use Deep Learning (AI)

Ready-To-Use Deep Learning algorithm was used for identification and segmentation of nuclear signals (i.e., DAPI or Iridium). Cell boundaries were 'estimated' based on dilation. No optimization required.

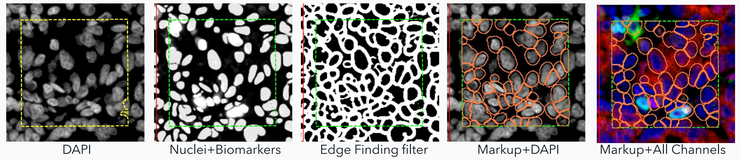


DAPI | Nuclei – DL Feature | Boundary – DL Feature | Markup+DAPI | Markup+All Channels

Advantages: Ready-To-Use; robust to morphological diversity (big and small nuclei)
Disadvantages: Trained on nuclear signals, cell boundaries still need to be estimated

Adapted Deep Learning (AI+)

The Ready-To-Use Deep Learning algorithm was adapted to include signals from biomarker channels that could assist with accurately delineating cell boundaries, defined as Nuclei+Biomarkers. An edge finding filter was then applied to the Nuclei+Biomarkers to approximate the cell border. Some optimization is required, but it is much less than in the Traditional Image Analysis method noted above.



DAPI | Nuclei+Biomarkers | Edge Finding filter | Markup+DAPI | Markup+All Channels

Advantages: Ready-To-Use; robust to morphological diversity (big and small nuclei) and includes biological signal for accurate detection of cell boundaries
Disadvantages: Requires some optimization

SCORING & PERFORMANCE RESULTS

$$Dice\ Coefficient = \frac{2*TP}{FN + (2*TP) + FP}$$

$$Jaccard\ Index = \frac{TP}{TP + FN + FP}$$

$$Sensitivity = \frac{TP}{TP + FN}$$

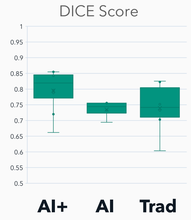
$$Precision = \frac{TP}{TP + FP}$$

TP - true positive
TN - true negative
FP - false positive
FN - false negative

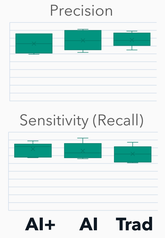
We compared computer performance to ground-truth human annotation using industry standards of:

- DICE score
- Precision
- Sensitivity

Akoya Vectra Polaris 8-plex Lung Cancer

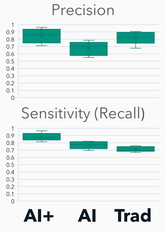


- DICE Scores for AI+ method show improved performance vs Ground Truth
- Precision and Sensitivity (measures of object detection) are similar across methods



Fluidigm Hyperion 13-plex Spleen

- DICE Scores for all methods show similar performance vs Ground Truth
- Precision and Sensitivity (measures of object detection) are roughly equal across methods



CONCLUSIONS

We developed a flexible AI-based strategy that enables the most complete segmentation of cells in varying multiplex images.

- All algorithms performed at a high-level using industry standards including DICE Scores, Precision and Sensitivity.
- The Adapted Deep Learning algorithm showed highest concordance with Ground Truth annotations.
- Performance differences across instruments could be attributed to resolution differences of the instruments, the number of biomarkers in the panel, and differences in images tissue and disease state.
- Despite annotation differences among human observers, the algorithms perform consistently across the image set.

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