Semi-Automatic Segmentation Workflow for Multiplexed Ion Beam Imaging

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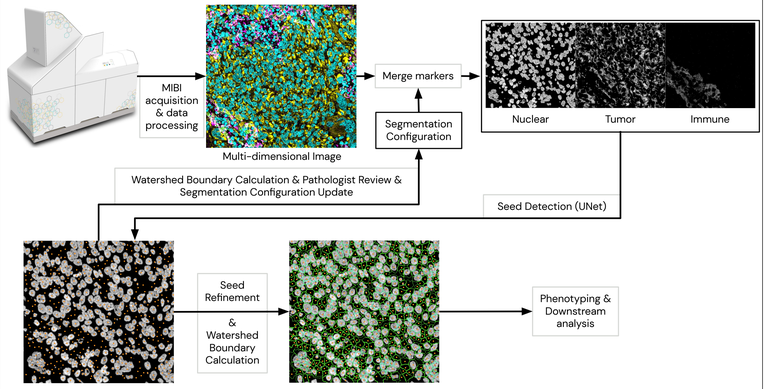
Introduction

- Multiplexed Ion Beam Imaging (MIBI) uses secondary ion mass spectrometry to image antibodies tagged with metal ions.
- High-resolution & highly multiplexed imaging (~40 biomarkers).
- Single cell segmentation of MIBI data allows downstream quantification and spatial mapping of cell populations in the tissue microenvironment.
- Need to reduce high-dimensional data into labels (individual biomarker channels to nuclear, tumor, immune, etc.)
- It is important to consider edge cases, e.g., such as enucleated cells.
- **We present a semi-automatic workflow for cell segmentation that uses a highly-configurable tool along with machine learning to estimate initial cell boundaries, and a manual refinement stage to improve accuracy.**

Methods: Segmentation Pipeline



Merging channels into groupings

- Challenge: Heterogeneous morphology and variability of marker expression.
- We merge markers into “groupings” such as “nuclear”, “tumor”, “immune”.
- These groupings are merged again to generate single-channel image.
- Cell boundary constraints are placed on these groupings.
  - e.g. A cell should not have immune and tumor markers.
- A “segmentation configuration” determines the rules for merging operations.
  - Which markers to use, how to merge, boundary smoothness, seed density.

Generation of seed points and initial boundaries

- The single merged channel is fed through a Neural Network (Unet).
- Output of UNet is a probability map for the center of each cell.
- Watershed segmentation is used to determine cell boundaries.

Review of Segmentation Configuration

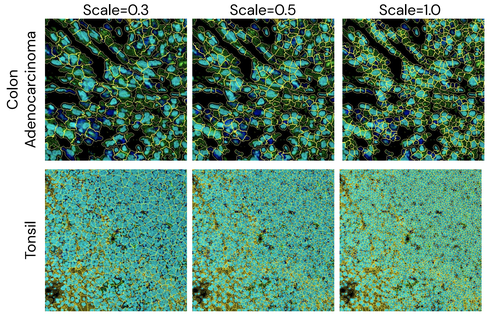
- Generated seeds and boundaries are reviewed by an experienced pathologist
- Segmentation configuration is updated to get to the “best” initial boundary estimates.

Seed Refinement

- Seed locations are manually refined using in-house software.
  - An analyst can move, add or delete seed points.
- New boundaries are generated using refined seeds.

Effect of Seed Prediction Scale

- After merging and before seed detection, the single channel can be rescaled.

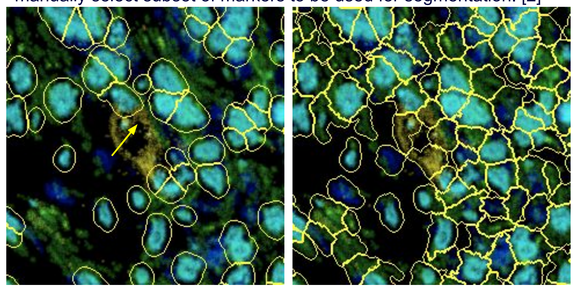


The figure on the left shows the effect of scaling before the single-channel merged image is processed to detect seed locations. For samples containing larger cells (first row), a lower scale factor is required to reduce cell splitting, whereas if the cells are smaller (second row), a larger scale factor is needed to prevent undersegmentation. Legend: Cyan: nuclear (dsDNA+Ki-67), blue: immune (CD14 + CD163 + CD68 + CD45), yellow: tumor (Keratin), green:vimentin. Shown images are crops of originals.

- It is possible to automatically determine the scale using intermediate features in UNet [1].

Enucleated cells

- Enucleated cells can be observed for larger cells when the nucleus is out of the imaged section.
- Using only nuclear markers results in those parts of the tissue to be missed in downstream analysis.
- Due to high variability of the types of markers that can be imaged using MIBI, it is necessary to manually select subset of markers to be used for segmentation. [2]



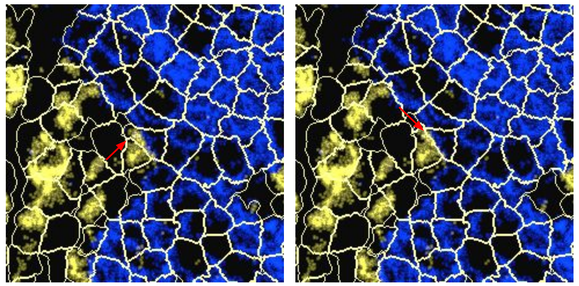
The left image only used dsDNA as input to watershed boundary calculation, whereas the right image uses nuclear, tumor, immune and Vimentin as input. Note that using only nuclear markers results in the rumor region to be missed since it has no nuclear signal (yellow arrow). On the right, the tumor region is caught, but it is oversegmented due to the void in the middle.

Conclusions & References

- A semi-automatic workflow for segmentation of MIBI data is presented.
- The review process ensures that the initial set of boundaries are as accurate as possible.
  - The following refinement step takes a minimum amount of manual labor.
- Can handle edge cases:
  - Nuclear signals are absent
  - Membrane markers are variably expressed.
- Future work:
  - Using output of UNet as input to watershed.
  - Workflow to automatically utilize refined seed points to improve ML models.
  - Project-specific ML model refinement.

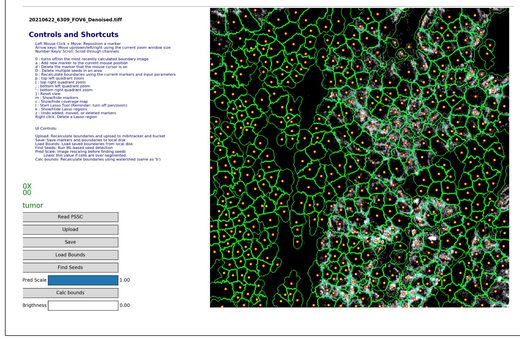
Celltype Boundary Constraint

- Celltype boundary constraints are introduced to prevent erroneous marker assignment to a cell.
- The figure below shows a case where the same cell contains both tumor and immune markers(yellow arrow)
- The boundary is moved in between the tumor and immune regions after celltype boundary constraints are imposed.



- Celltype boundary constraints are part of the segmentation configuration.
- This boundary constraint was applied using:
  - `boundary = skeletonize(tumor * immune)`
  - `watershed_image = watershed_image - w * boundary`
  - skeletonization creates a one-pixel thick boundary from a grayscale image
  - “w” is an adjustable weight parameter
- It is also possible to fix such spillovers during classification. [3]

User interface



- We developed a simple user interface in Python that an analyst can use to refine seed points.
- User can add/delete/move seed points, browse different channels, calculate boundaries.
- As a last resort, it is also possible to manually draw boundaries.

References

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[2] Greenwald, N. F., Miller, G., Moen, E., Kong, A., Kagel, A., Fullaway, C. C., … Valen, D. Van. (2021). Whole-cell segmentation of tissue images with human-level performance using large-scale data annotation and deep learning. BioRxiv, 2021.03.01.431313. https://doi.org/10.1101/2021.03.01.431313

[3] Bai, Y., Zhu, B., Rovira-Clave, X., Chen, H., Markovic, M., Chan, C. N., … Jiang, S. (2021). Adjacent Cell Marker Lateral Spillover Compensation and Reinforcement for Multiplexed Images. Frontiers in Immunology, 12, 2510. https://doi.org/10.3389/FIMMU.2021.652631