Development of a high-throughput image processing pipeline for multiplex immunofluorescence whole slide images at scale

Abstract #6616

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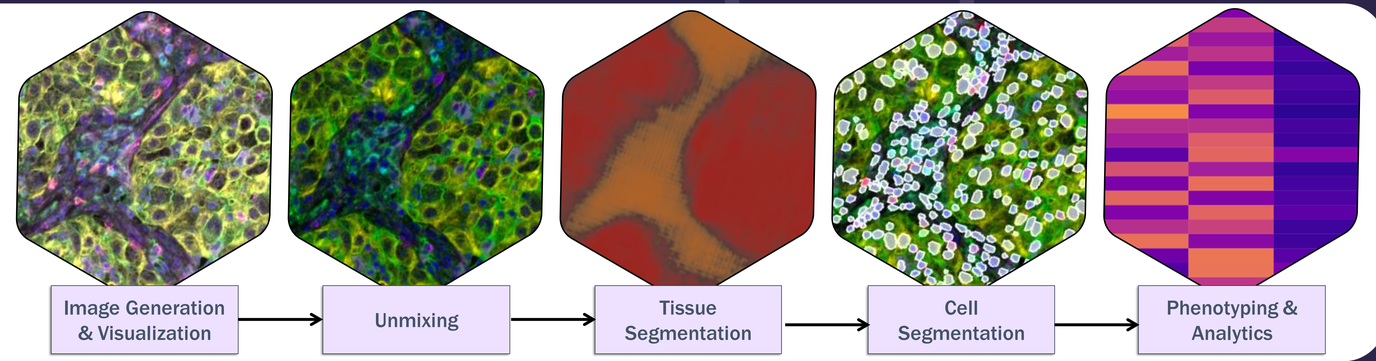
STUDY BACKGROUND

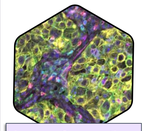
- Fluorescent imaging technologies now allow for multiplex immunofluorescence (mIF) of up to 100 targets on a single slide. However, the ability to quantitatively analyze the resulting data, especially on whole-slide images (WSI), is limited by scalability and reproducibility.
- Currently used platforms for segmenting cancer cells and nuclei involve segmentation algorithms that are hand-tuned on individual fields of view, making these methods subjective and difficult to replicate.
- To this end, we sought to develop an end-to-end workflow for WSI mIF data in cancer, from raw images to cell-level features (Fig. 1), using state-of-the-art deep learning models for tissue, cell, and nuclei segmentation.

END-TO-END MULTIPLEX IMAGE ANALYSIS

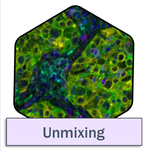
Figure 1. Workflow for mIF image segmentation and analysis.



METHODS AND RESULTS

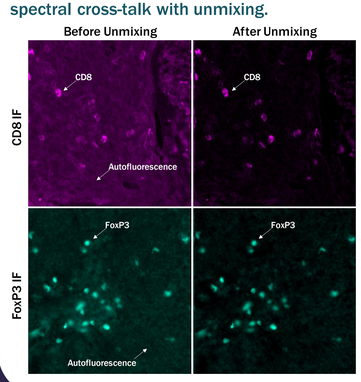


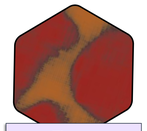
mIF was performed using the Akoya 6-plex Lung IO panel (CD8, FoxP3, CD68, PD-1, PD-L1, and pan-cytokeratin) with a DAPI counterstain on clinical NSCLC specimens obtained from commercial sources (N=41). This panel was chosen due to the mix of nuclear, cytoplasmic, and membranous target localizations and current widespread laboratory use. Slides were scanned using the Akoya PhenoImager HT.



To remove bleed-through between fluorophores and autofluorescence, mIF WSIs were linearly unmixed based on a reference single-stain matrix that was generated by selecting pure stain regions in mIF images (Fig. 2).

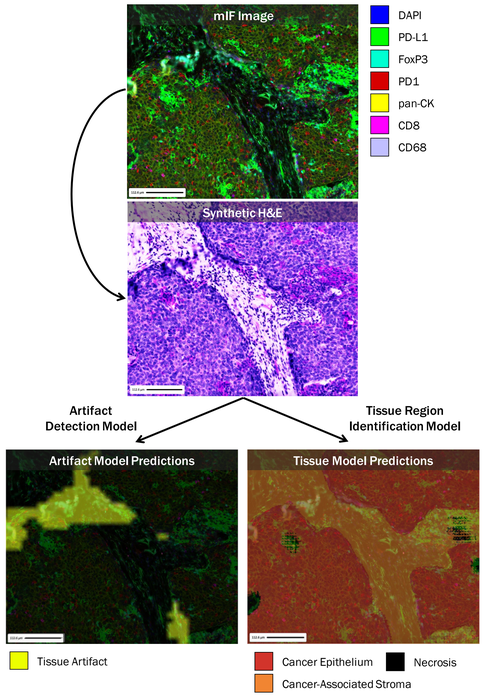
Figure 2. Removal of autofluorescence and spectral cross-talk with unmixing.

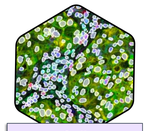




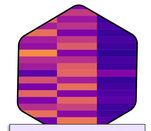
For each mIF image, a synthetic H&E image was generated by mapping the DAPI signal to a hematoxylin stain vector and a composite of all other channels to an eosin stain vector (Fig. 3). Convolutional neural network models previously trained on H&E images for artifact detection and tissue region identification were then deployed on synthetic H&E images.

Figure 3. Use of synthetic H&E for tissue segmentation.



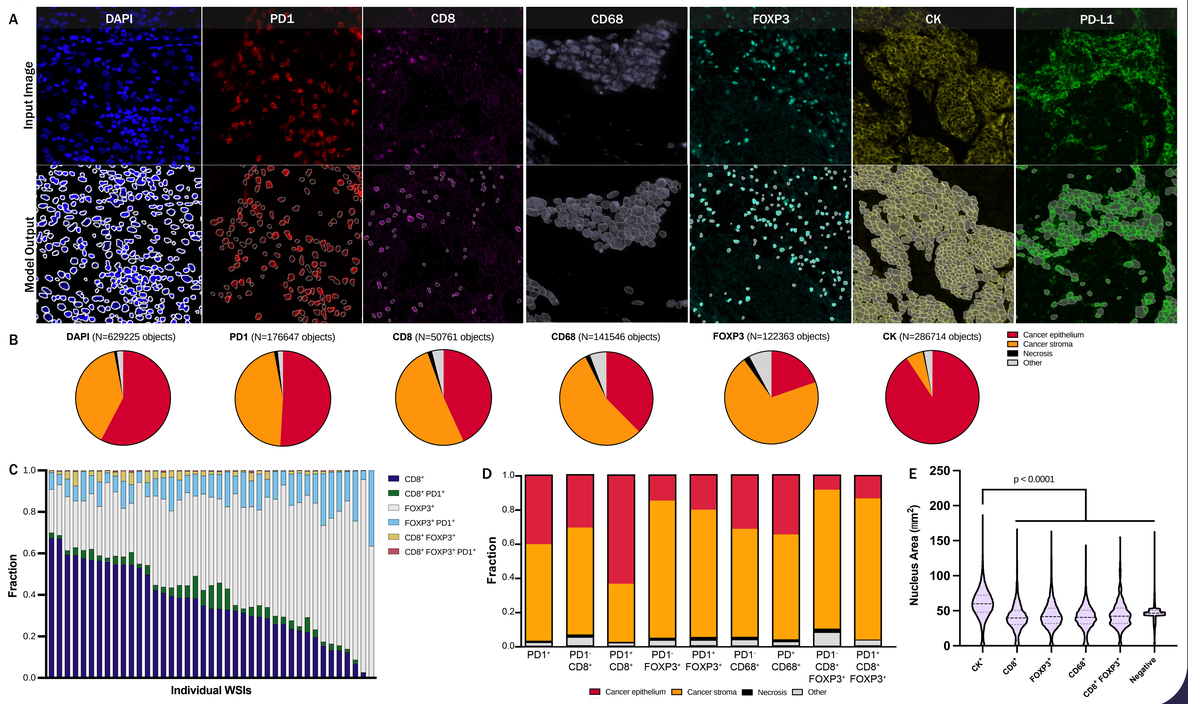


For each unmixed channel representing a single antibody or DAPI signal, expert annotations were used to train deep learning models for cell detection and segmentation. Examples of input single-channel images and model predictions are shown in Fig. 4A.



Analyses were performed based on segmented object location and size. The tissue region distribution for cell nuclei and PD1$^+$, CD8$^+$, CD68$^+$, FOXP3$^+$, and CK$^+$ cells in an example WSI are shown in (Fig. 4B). The relative frequencies of segmented cell types at the whole-slide level shows distinct inter-tumoral heterogeneity in lymphocyte phenotypes (Fig. 4C), while tissue-region analysis confirms that PD1$^+$ CD8$^+$ cells are most likely to be tumor infiltrating (Fig. 4D). Cell-type-specific nuclei areas were also calculated (Fig. 4E), confirming enlarged cancer (CK$^+$) nuclei compared to other cell nuclei ($p<0.0001$; Kruskal-Wallis test).

Figure 4. Performance of mIF-derived segmentation models and examples of downstream analytics.



CONCLUSIONS

- We developed a robust workflow to perform comprehensive image analysis on mIF WSIs at scale, including unmixing, tissue segmentation, and cell segmentation.
- Features extracted from the models developed herein can be used to perform cell-level analytics across a WSI at scale.
- Approaches such as these can improve the scalability and reproducibility of complex mIF image analysis. Adoption of this approach and incorporation into clinical trial workflows in oncology may improve biomarker identification and endpoint analyses, potentially improving patient outcomes.

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