Single-cell phenotyping using optical imaging and artificial intelligence

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Background. In recent years, the popularity of single cell analysis has been driven by the consensus that tissue level function can be better understood by mapping individual cell diversity rather than through bulk-tissue analysis. The utility of this is most profound in cancer biology, where pinpointing clonal lineages and morphological changes cell-by-cell gives unprecedented insight about the disease. Central Nervous System tumors in particular have immense cytoarchitectural complexity with intricate microvascular networks and numerous cell types. There is a need to spatio-anatomically profile these cells in their natural environment to better identify the role of various contributors to tumorigenicity and disease progression. Unfortunately, current single cell analysis methods have been limited by tissue architecture disruption, scalability, immature analytical algorithms and lack of real-world data. The advent of Stimulated Raman Histology (SRH) has offered a way to rapidly image tumor biopsy samples and discern the concentrations of lipids, proteins and other molecular components at a subcellular spatial resolution without requiring histologic staining. Clinical SRH microscopes are already being used by neurosurgeons to intraoperatively image brain tumor biopsies at the bedside. We propose using SRH and vision-based AI models for rapid *in situ* cell phenotyping of human brain tumor biopsies. Vision-based AI algorithms have been valuable in processing large histologic images and detecting information such as tumor type, grading and even molecular markers. Applying these models to SRH brain tumor biopsies can allow for real-time tumor microenvironment analysis for clinical decision making at the bedside.

Methods. SRH images are captured by shining a laser on a tissue sample and measuring intensities at specific wavelengths. For brain tissue, the 2845 cm-1 wavenumber (CH_2 bond/lipid channel) and 2930 cm-1 wavenumber (CH_3 bond/protein channel) provide sufficient contrast for identifying neuronal structures. The two channels are computationally pre-processed into a 3-channel RGB image that mimics a virtual hematoxylin and eosin (H&E) stain. The image is then divided into 300 by 300 pixel patches and human-annotated with cell segmentations using a web-based SRH labeling tool adapted from the open-source Django-Labeller project on GitHub. A Mask R-CNN and Faster R-CNN model with a ResNet-50 Feature Pyramid Network backbone pre-trained on Microsoft COCO dataset was fine-tuned on 50 annotated SRH patches of gliomas and validated on 1,081 patches. Final predictions from the model used a non-max suppression algorithm to remove overlapping cell bounding boxes with >20% area and kept predictions with a confidence score above 0.80. All tissue samples used in the study were sourced from consented University of Michigan Neurosurgical patients.

Results.

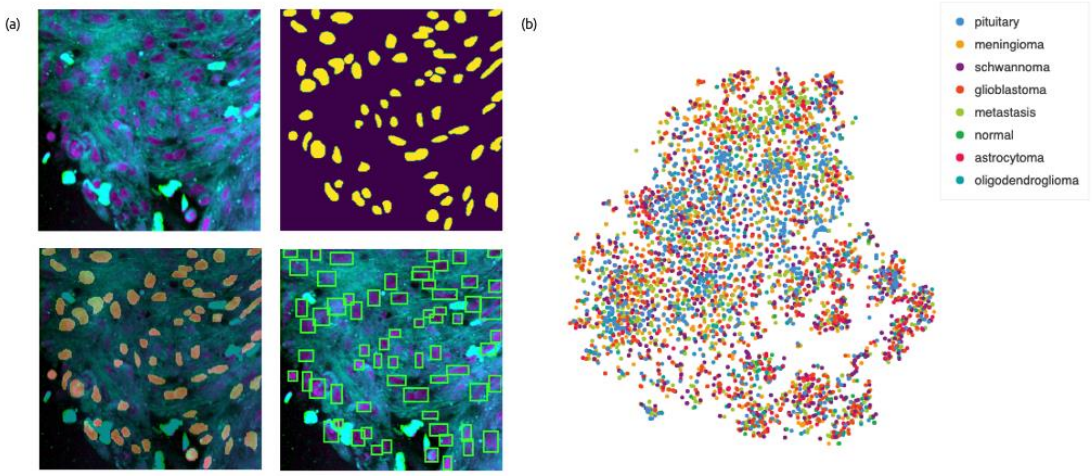

Figure 1. (a) Shows a raw SRH image of a glioma after pre-processing (top left) followed by the predicted masks and bounding boxes from the Mask-RCNN model. (b) t-SNE embeddings of the FC-7 layer of the Mask RCNN from ~4,000 segmented cells from 1,026 SRH patches spanning 7 brain tumor types.

The Mask R-CNN and Faster R-CNN had a mean average precision (mAP) of 0.90 and 0.88, respectively. Empirically, cell segmentations produced by the Mask R-CNN showed robust generalization to various cell morphologies and CNS tumor pathologies despite a small training set. A t-SNE of the activations from the FC-7 layer within the Mask-RCNN's classification head revealed underlying phenotypic clusters on a SRH dataset representing 7 different brain tumor types.

Conclusion. The preliminary results from this approach is the first step for automatically analyzing brain tumors at a cellular-level without the need for special staining. Ongoing work aims to use unsupervised learning to investigate cell morphologies distinct to the major CNS tumor types. We also plan to grow the annotated SRH dataset using model predictions and a physician-in-the-loop active learning method. This platform will provide researchers and clinicians a better way to objectively characterize the tumors using AI and opens the door for tailoring diagnostic and therapeutic approaches under the principles of precision oncology.