**Background**

Comprehensive biomarker profiling from clinical samples with limited serial sections is not feasible using a traditional immunohistochemistry (IHC) assay. Standard classical deconvolution techniques some biomarkers on a single slide fail due to high autofluorescence (AF), decreasing the window for visualization. Due to high levels of AF, a threshold is needed to control for signal-to-noise ratio (S/N) to be accurately detected. This results in a threefold decrease in average per-study analysis time. A fully automated approach that removes much of the remaining manual labor and allows for complex spatial analytics and is capable of answering complex queries such as: 1. Are certain immune biomarkers excluded into the tumor region? 2. Are certain single-cell activators (OX40, ICOS, GITR) and suppressors from the tumor region? 3. Are certain immune biomarkers absent or reduced in number when compared to more traditional computer vision methods requiring high levels of parameter fine-tuning that were employed in prior work. 

**Workflow Steps**

The framework consists of seven major steps:

1. **Manual annotation of a small subset of each biomarker channel**
2. **Classification of entire dataset to delineate individual cells**
3. **Application of the trained network in (2) on the nuclear stain (DAPI) of the entire sample**
4. **Cell segmentation**
5. **Manual annotation of small area on pre-delineated cells**
6. **Application of trained network on manually selected cells**
7. **Visualization of data to ease understanding of the complex output**

The output consists of:

- Cell population maps labeled for every biomarker within a study panel
- Tumor segmentation masks
- Quantification files listing cell morphological features, intensity measurements, and classification results
- Summary tables for each biomarker and biomarker combination

Visualizations of data to ease understanding of the complex output

**Summary**

The benefits of using this Deep Learning framework are greatly felt through the improved efficiency and increased accuracy when compared to more traditional computer vision methods requiring high levels of parameter fine-tuning that were employed in prior work. A fully automated approach that removes much of the remaining manual labor and allows for complex spatial analytics and is capable of answering complex queries such as: 1. Are certain immune biomarkers excluded into the tumor region? 2. Are certain single-cell activators (OX40, ICOS, GITR) and suppressors from the tumor region? 3. Are certain immune biomarkers absent or reduced in number when compared to more traditional computer vision methods requiring high levels of parameter fine-tuning that were employed in prior work. 

**References**