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Profiling Cancer Biology

UltiAnalyzer.AI: An automatic and robust AI-driven tool for the quantification of multiplex immunofluorescence whole slide images

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Summary

Whole slide multiplex Immunofluorescence (mIF) scanning of tissue samples is becoming increasingly available, with various technologies enabling the production of massive amounts of data. Typically, the data for any one slide amounts to several gigabytes, representing millions of cells across multiple channels/biomarkers. Given the volume of data, it is important to process these datasets automatically and reliably. But while existing commercial tools such as Visiopharm™ or HALO™, along with open-source tools such as QuPath, facilitate these processing tasks by providing user interfaces running on a single machine or small cluster, they are oriented toward solving many digital pathology tasks interactively, requiring users to navigate the workflow for mIF slide analysis themselves. These solutions also exhibit significant limitations in terms of performance, scalability, throughput, and full automation. In this work, we introduce UltiAnalyzer.AI, part of the STARVUE™ platform that integrates a suite of artificial intelligence models with massively parallel cloud-based processing to provide high-quality whole slide mIF analysis with minimal manual interaction and very high throughput.
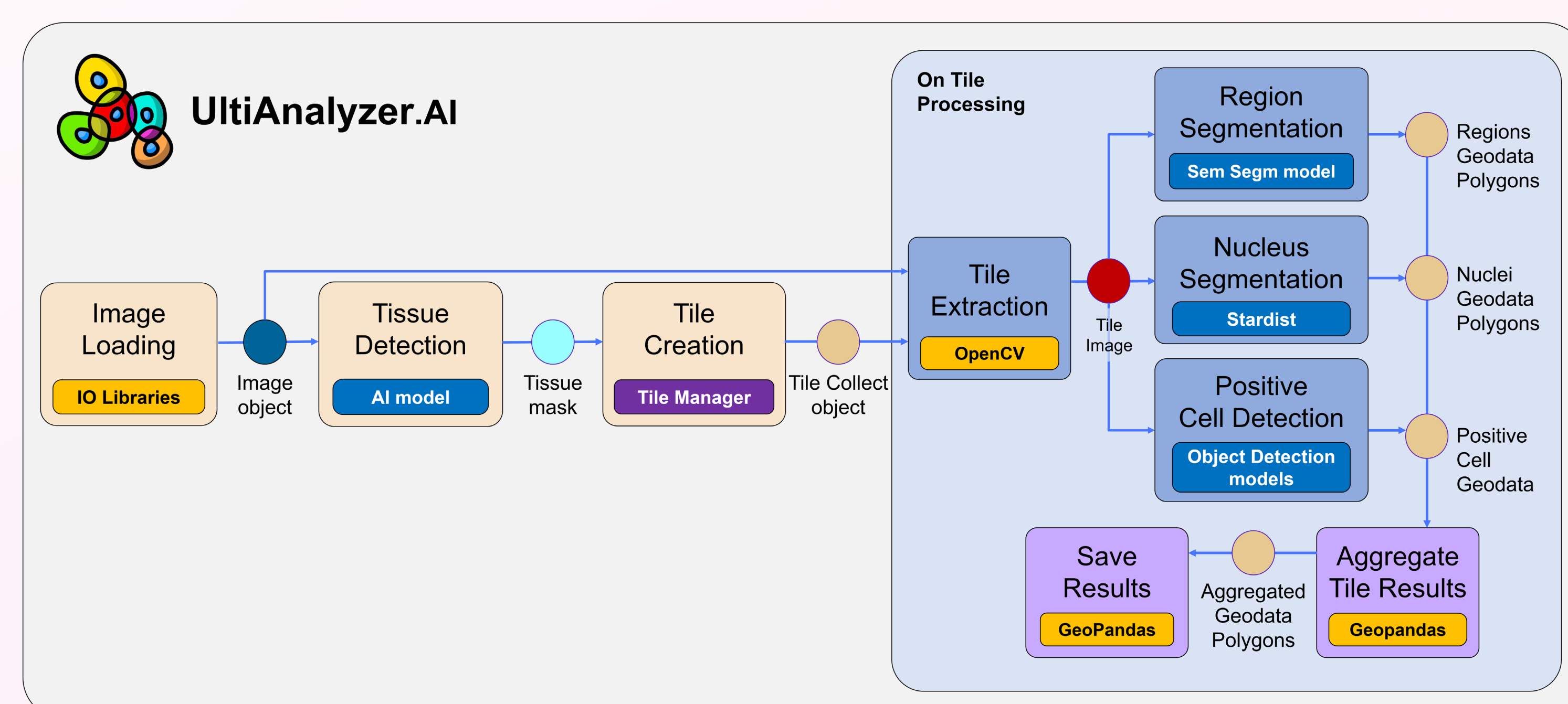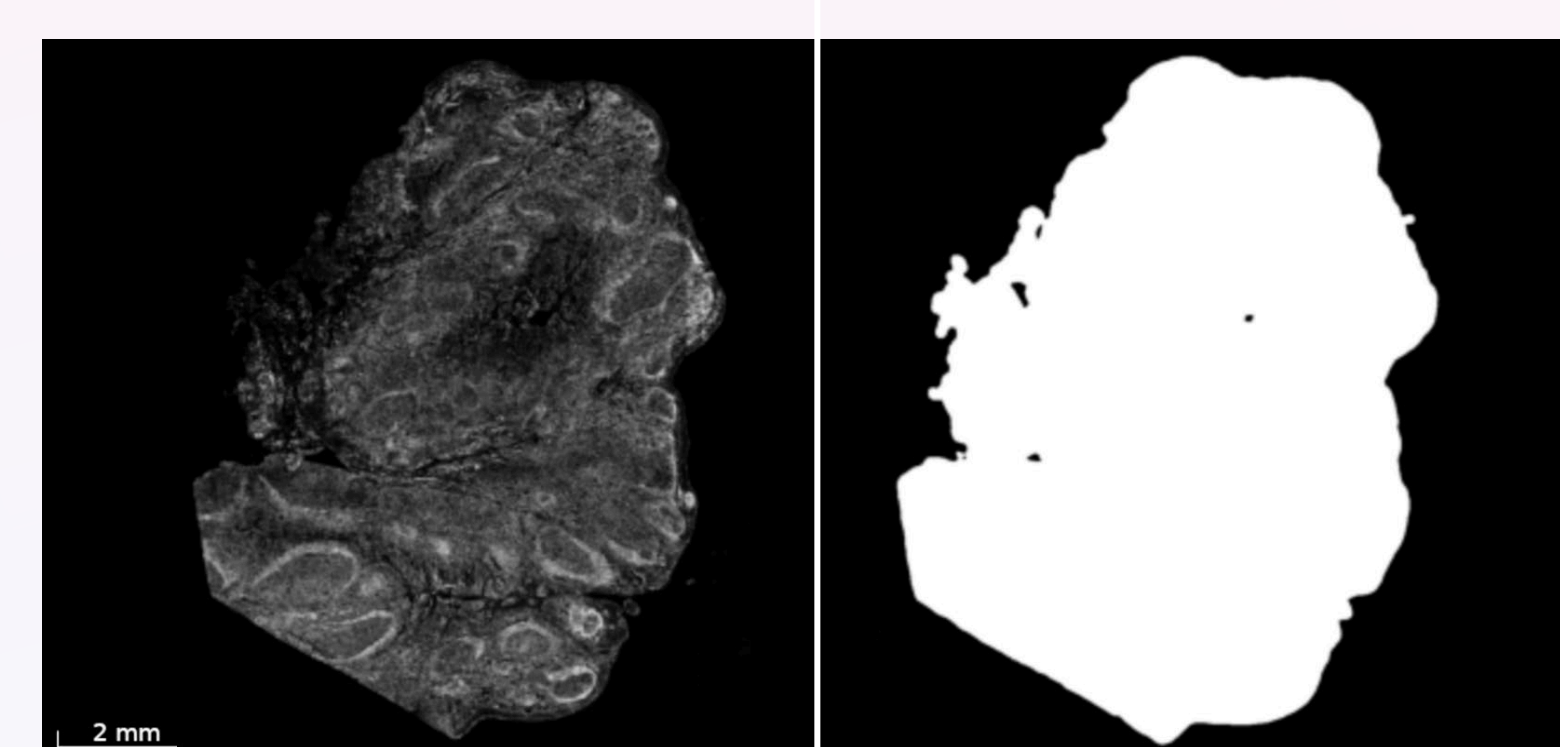
Figure 1. UltiAnalyzer.AI block diagram showing its main components.
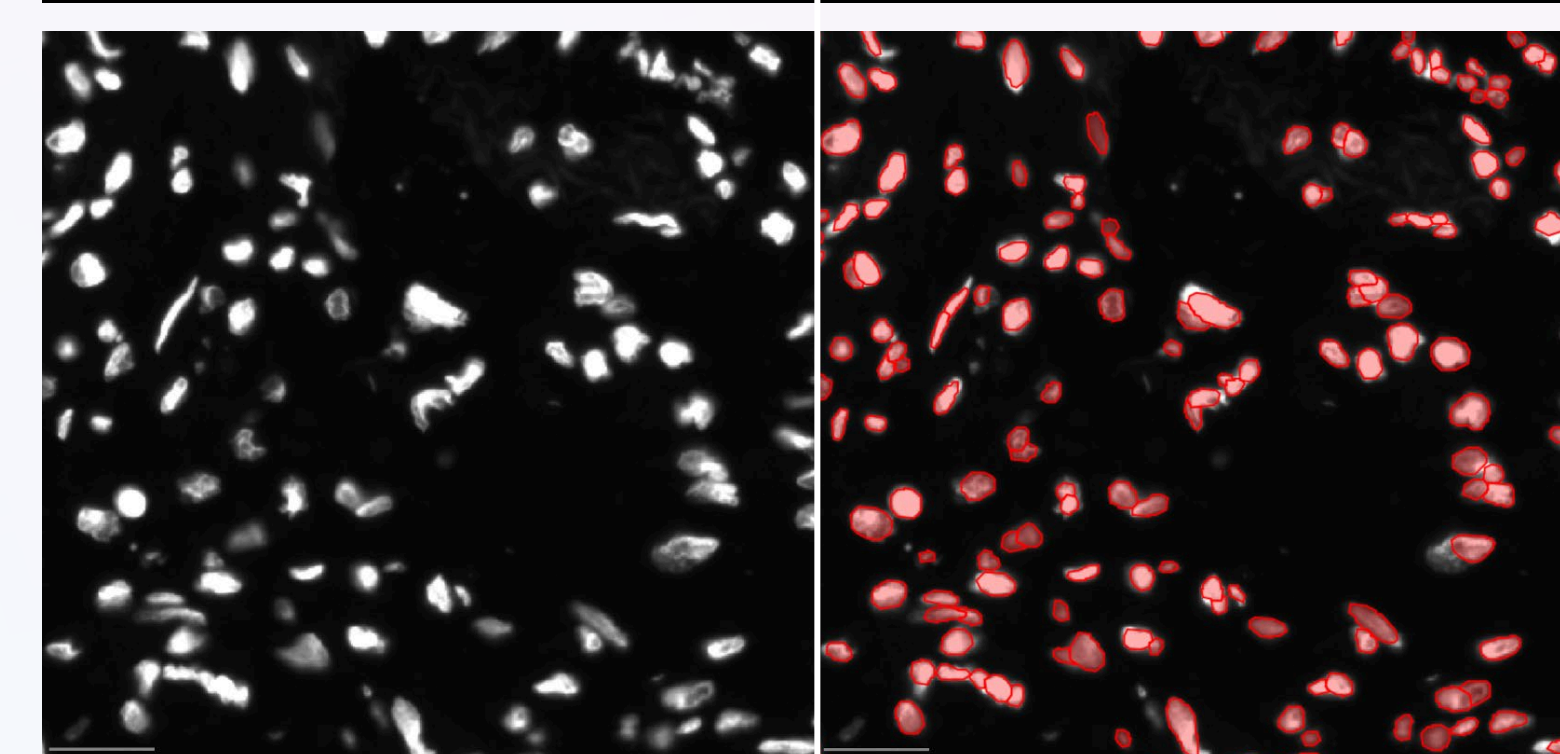
Methods

Tissue segmentation

- Operates on DAPI channel at low resolution
- Separates tissue from glass
- Uses a proprietary AI model
- Highly robust, fully automatic

Nucleus segmentation

- Operates on DAPI tiles at full resolution
- Uses open-source AI model: stardist
- Produces a polygon boundary for each nucleus
- Fully automatic (no parameter tuning required)

Region segmentation

- Segments regions from a target channel
- Typically used to find tumor regions using CK channel
- Using proprietary AI models
- Available solutions for CK, Sox10, Collagen
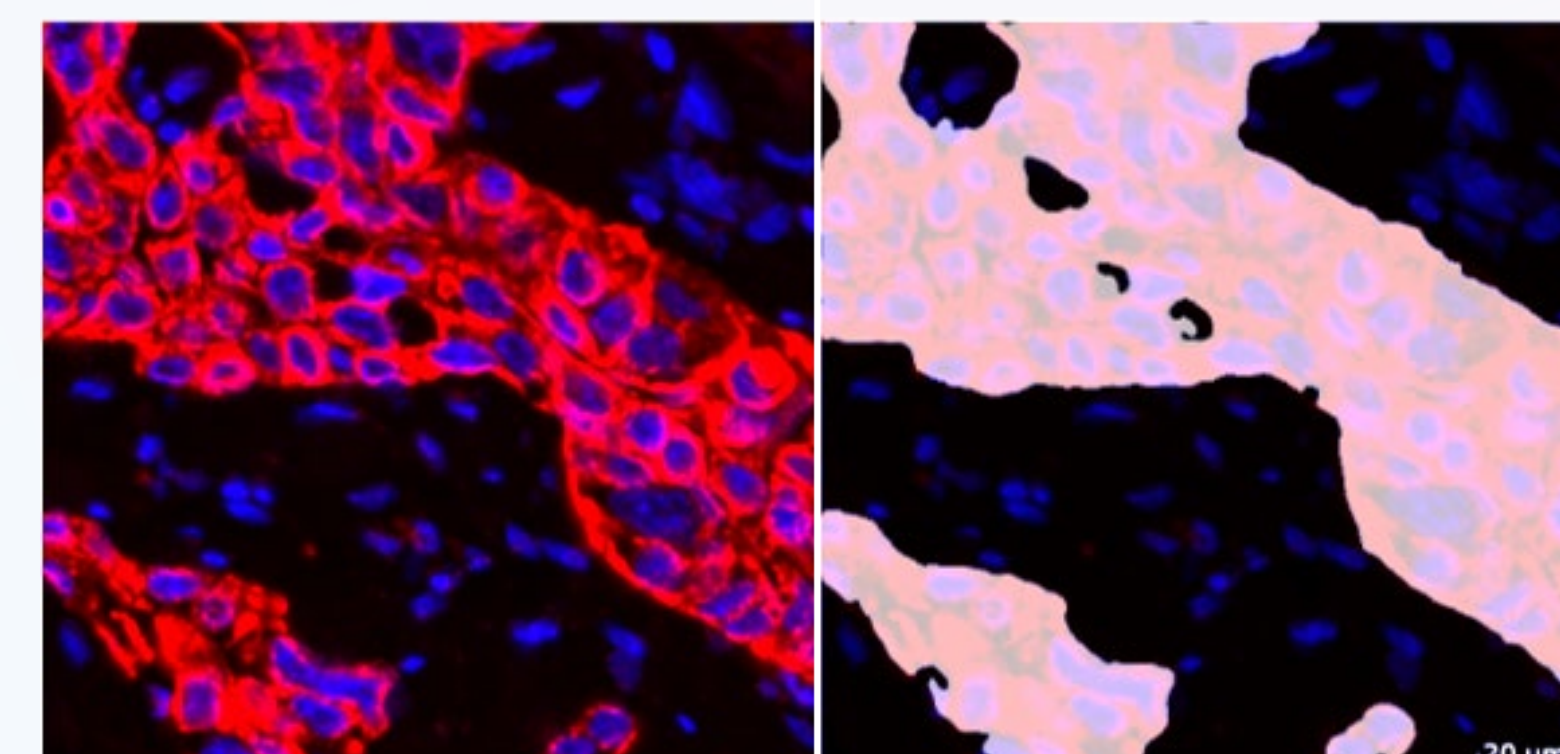
Positive Cell Detection

- Identifies positive cells on each channel
- Uses proprietary AI models (object detection)
- Uses state of the art detection transformer models (DETR) and other architectures
- Available solutions for a wide range of markers
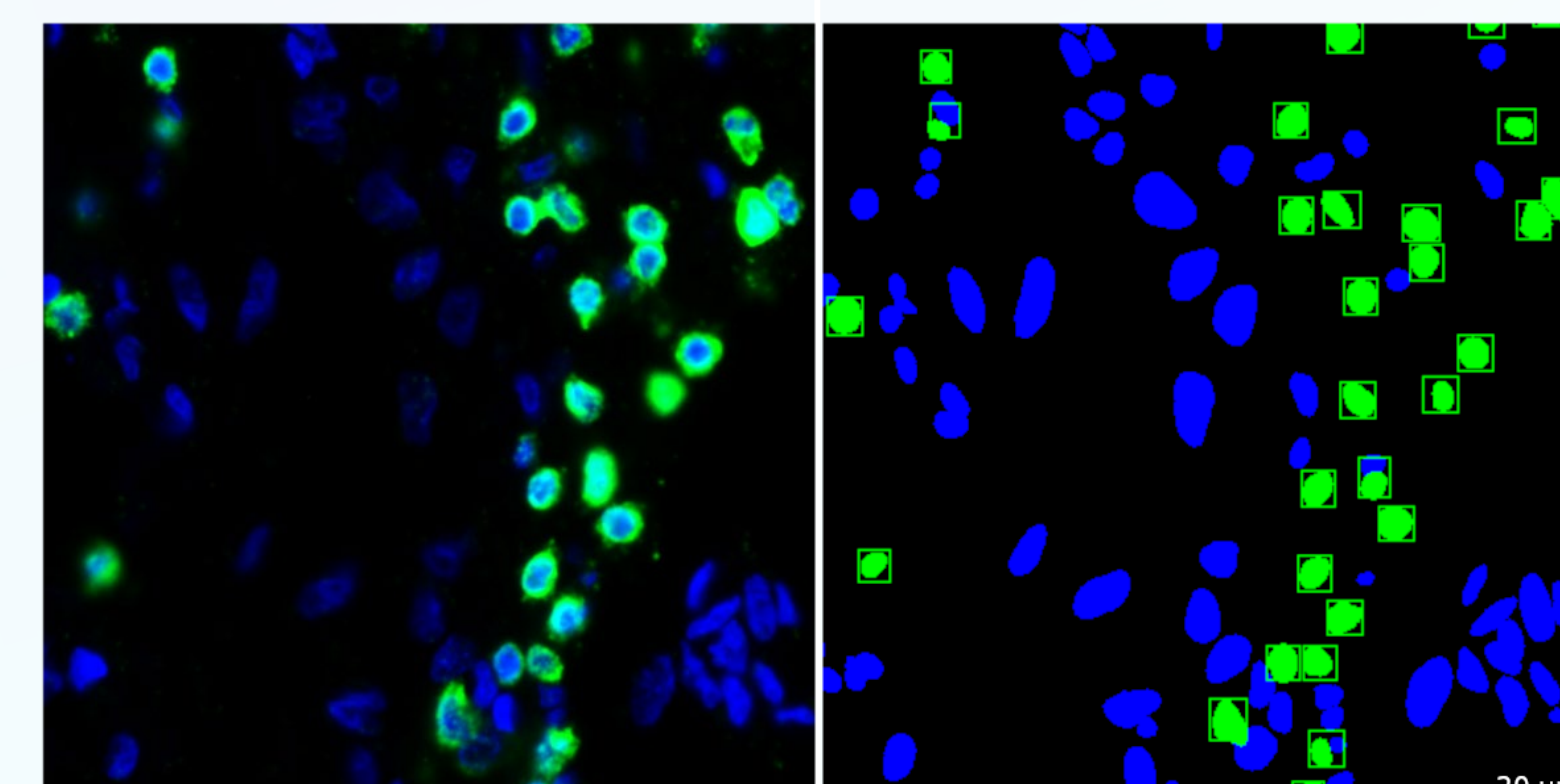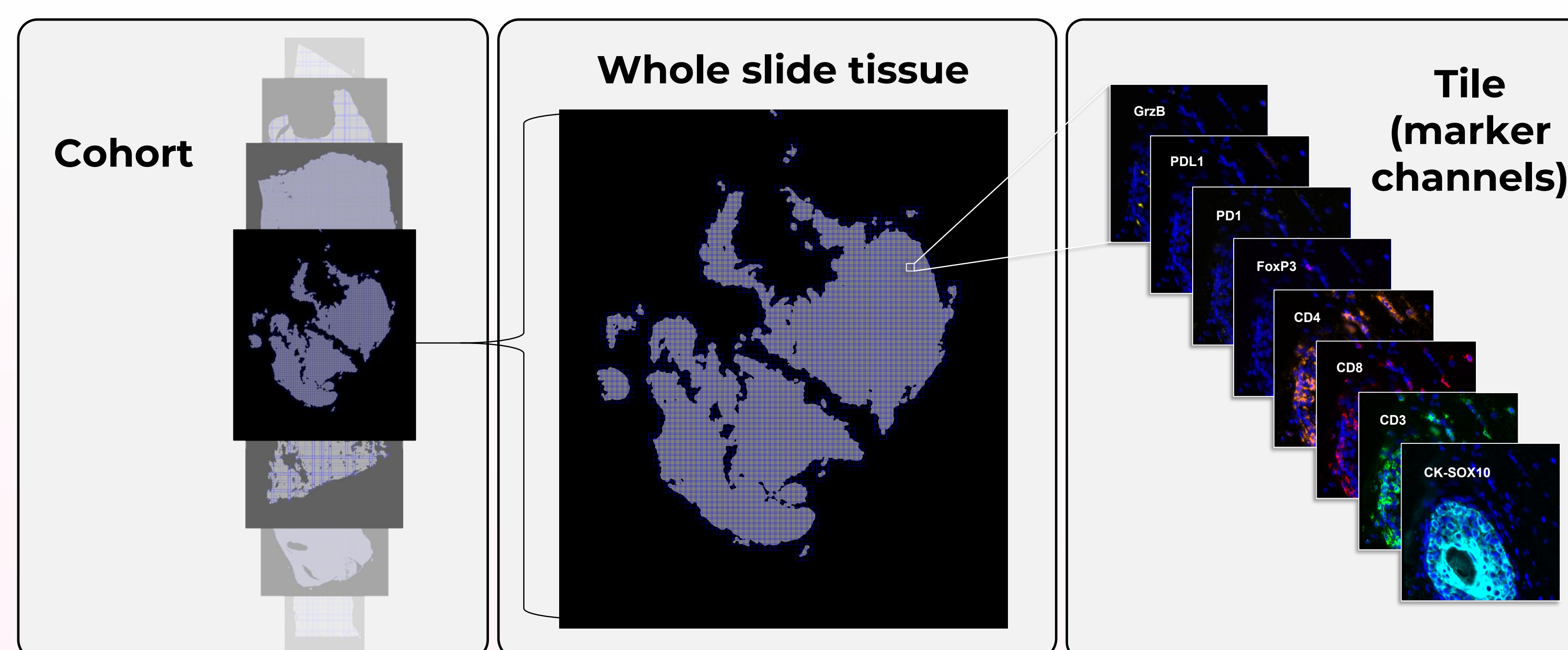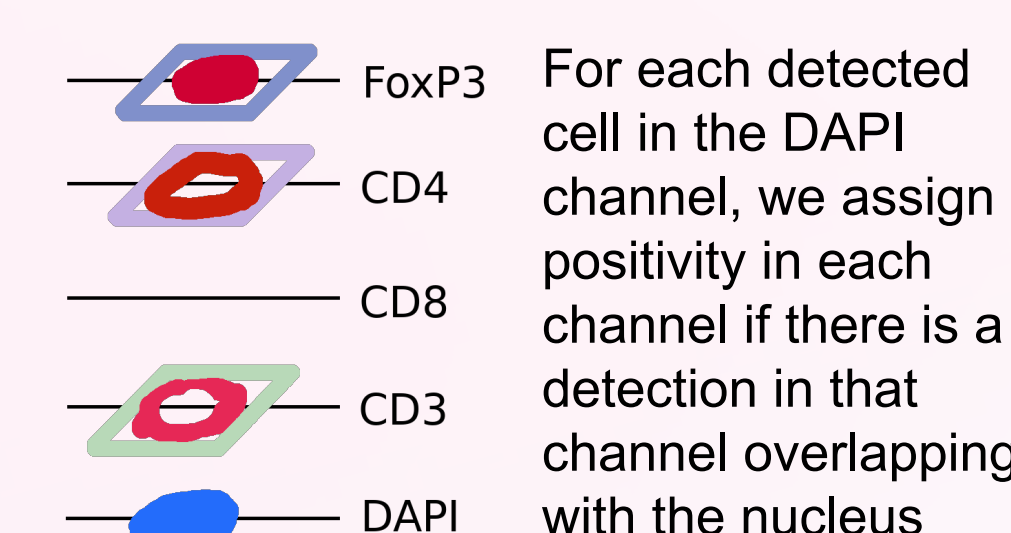

Overview of Working Levels

Figure 3. Levels of work for UltiAnalyzer.AI workflow. Cohort level: managed on the cloud, whole slide level: managed by UltiAnalyzer.AI slide processing, and tile level: managed by UltiAnalyzer.AI tile processing
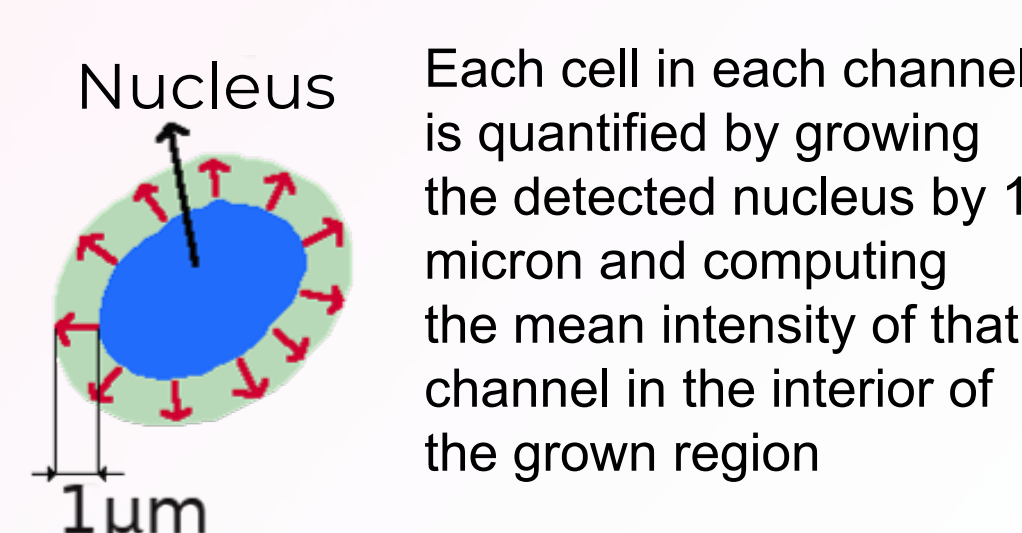
Tile processing

Slides are processed by tiles. Each tile contains an overlap region to avoid object cuts. Color coding is, green: cells inside the tile, yellow: cells outside the tile, gray: cells touching the tile border

Cell detection fusion of channels

For each detected cell in the DAPI channel, we assign positivity in each channel if there is a detection in that channel overlapping with the nucleus

Cell quantification

Each cell in each channel is quantified by growing the detected nucleus by 1 micron and computing the mean intensity of that channel in the interior of the grown region
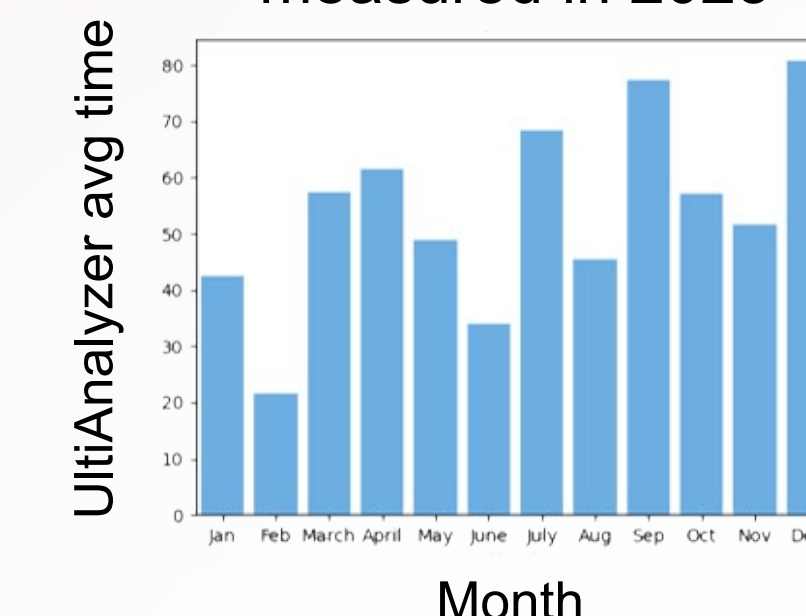
Performance and Scalability

Typical execution times for a normal resection with 8 channels is around 1 hour

Tile size (pixels)	Execution Time (sec)		
	4-Plex	8-Plex	12-Plex
512 x 512	0.807	1.691	2.203
1024 x 1024	1.694	4.961	-

Average execution times measured in 2023

UltiAnalyzer.AI can process more than 600 slides in parallel in the cloud

Statistics summary

- 4 semantic segmentation models
- 36 cell detection models
- 17 validated markers
- 46 different markers processed
- 191 batches processed
- 4378 total channels processed

Key Features

- High quality 16 bit image processing
- No thresholding (adapt to intensity changes)
- Fully scalable
- Fully automatic end-to-end workflow
- Can use same slide H&E to complement processing

Models Performance
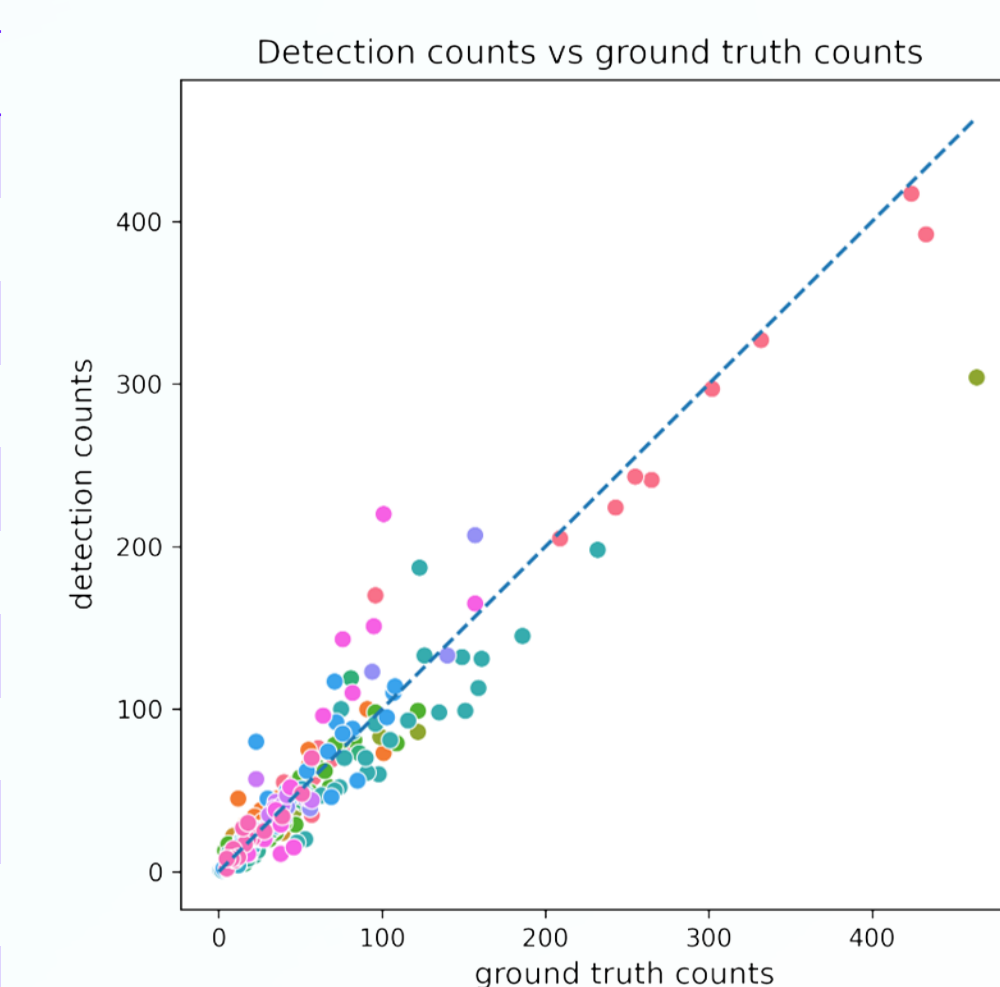
Marker	Lin's Corr	F1-score
CD3	0.990	0.916
CD8	0.861	0.861
PD1	0.870	0.806
Granzyme B	0.901	0.833
CD20	0.913	0.747
CD4	0.942	0.802
CD68	0.955	0.703
CD163	0.828	0.685
PD-L1	0.922	0.746
FoxP3	0.940	0.875
Ki67	0.834	0.803
CD11b	0.989	0.686
CD14	0.945	0.617
CD15	0.925	0.731

Model	Class	Accuracy
CK Model	Background	97.83
	CK	87.23
	Average	92.53
Sox10 Model	Background	91.39
	Sox10	88.22
	Average	89.80
Collagen Model	Background	97.18
	ColA1	97.11
	Average	97.14

- All detection models > 0.85 Lins' correlation
- Global Lin's Corr: 0.954
- Segmentation models are all above 89 % average accuracy
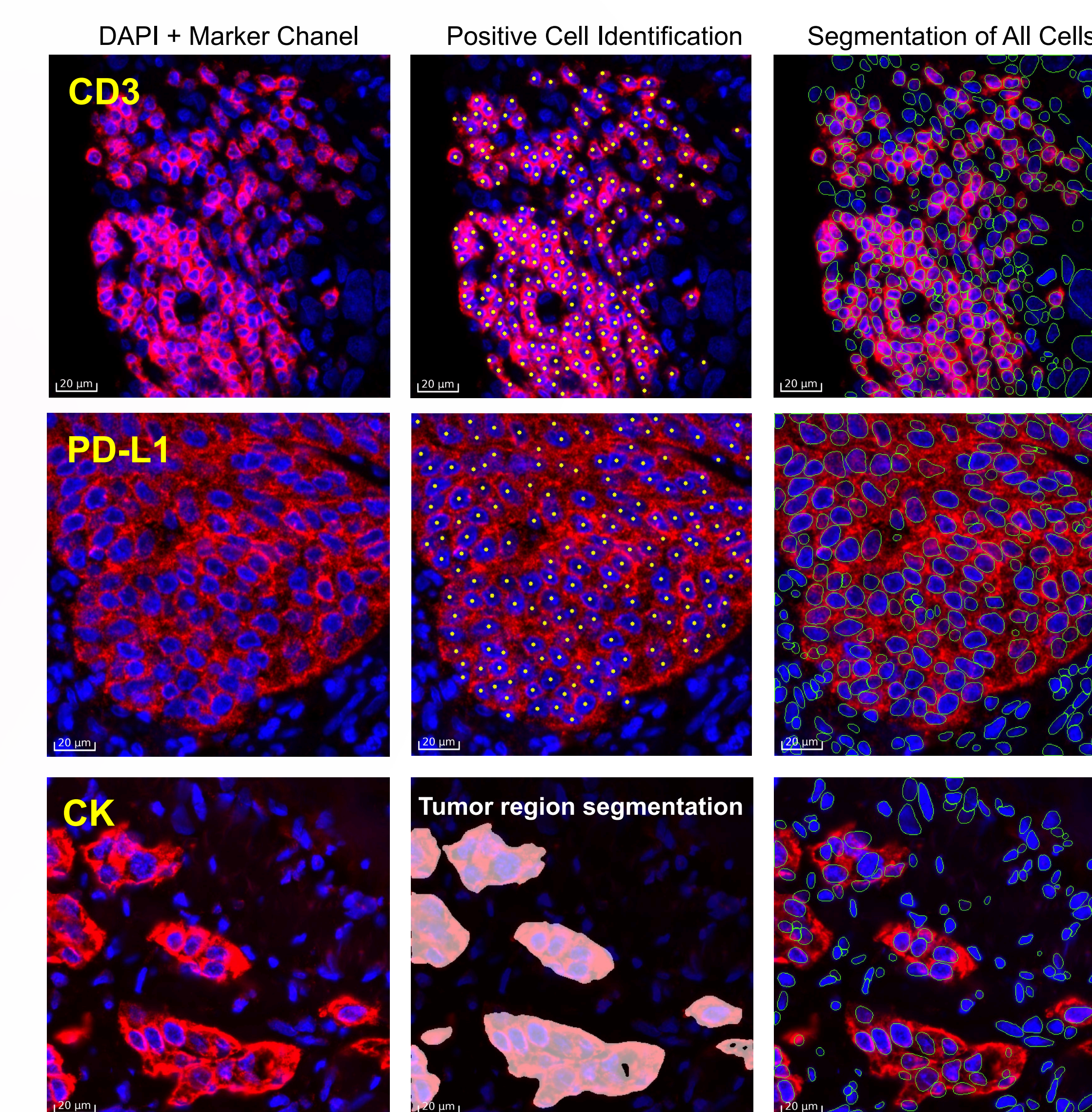
Results

Figure 4. Example tiles for cell marker detection (first and second row), and region segmentation (bottom row).

Comparison vs Visiopharm

UltiAnalyzer.AI results correlate very well with Visiopharm results (figure 5) and visually the results are also comparable (figure 6), where some markers are better handled by our models.
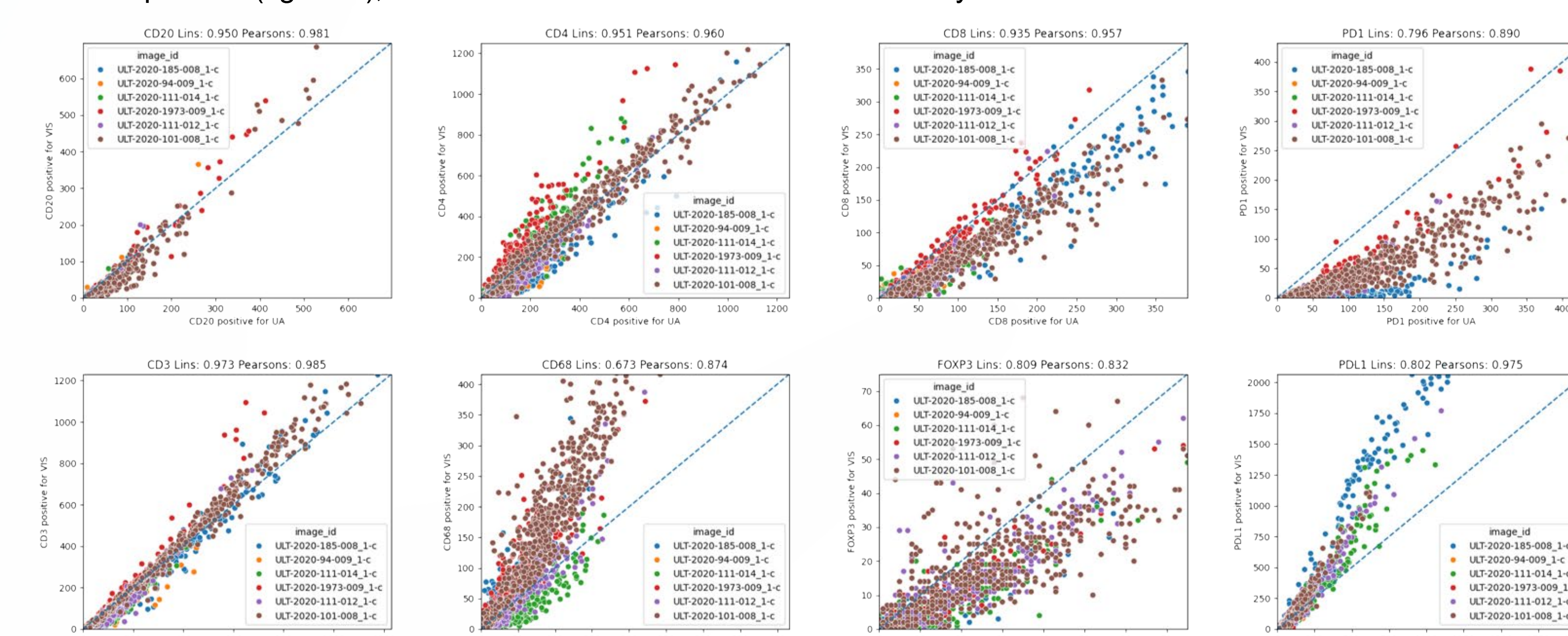

Figure 5. Correlation plots of positive counts between UltiAnalyzer.AI and Visiopharm on 8 markers. Each dot corresponds to a tile of 2048x2048 pixels in 20x magnification. Each color corresponds to a different slide.
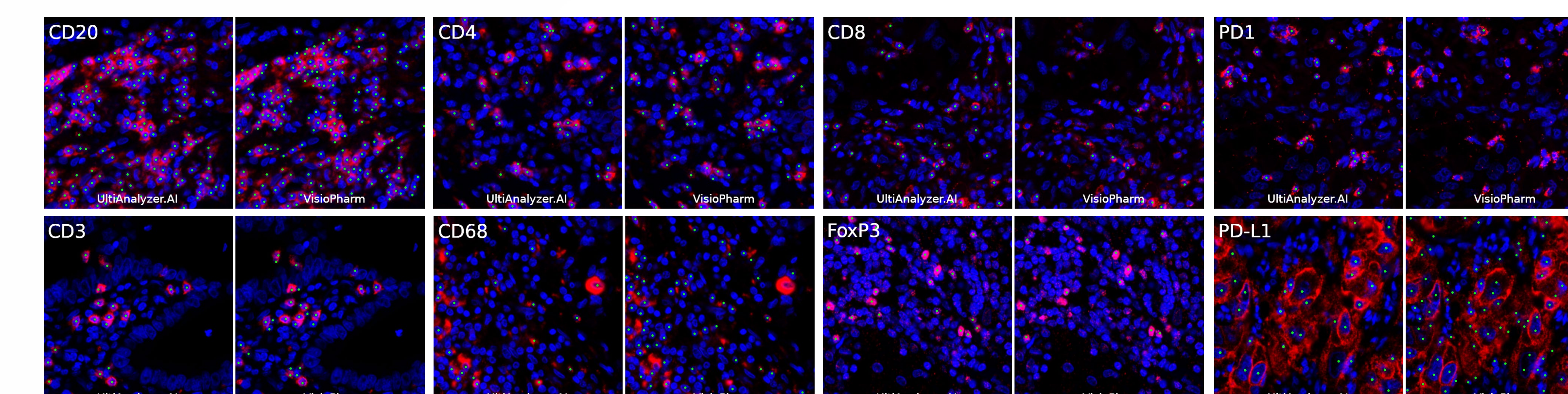

Figure 6. Example tiles comparing visually UltiAnalyzer.AI and Visiopharm (highly discordant tiles)

Conclusion

UltiAnalyzer.AI is a highly scalable AI enabled software that automatically processes whole slide multiplex IF images with high accuracy. It has been successfully tested in a variety of tissues and biomarkers and an extensive collection of AI models are now available. As part of STARVUE™ platform, it is to our knowledge the first cloud-based solution that can perform high accuracy multiplex IF analysis of whole slide tissue specimens at scale.