Celtix: An End-to-End Spatial Analysis Platform for High-Plex Tissue Imaging with Application to Head and Neck Cancer

Bassem Ben Cheikh[1], Meg Donovan[2], James Monkman[3], Arutha Kulasinghe[2, 3]

[1] Tixomics, Los Angeles, CA, USA; [2] Wesley Research Institute, Auchenflower, Queensland, Australia; [3] The University of Queensland, Brisbane, Queensland, Australia

Abstract

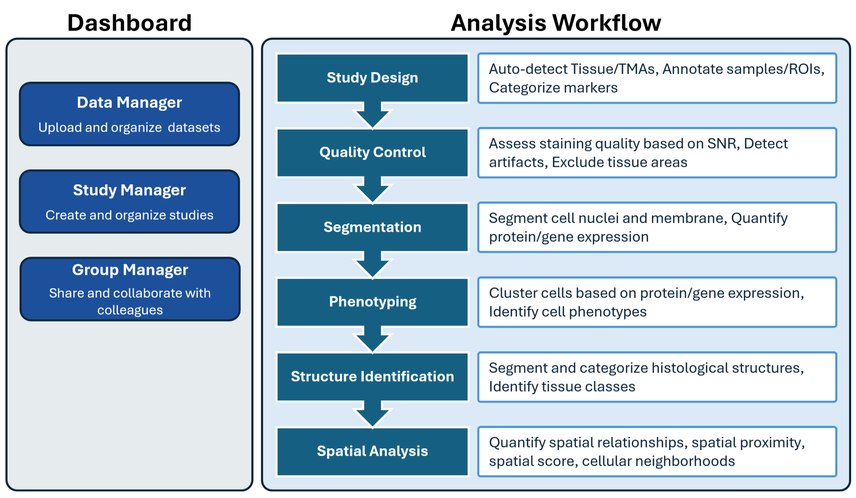
High-plex spatial proteomics produces large, complex datasets that remain challenging to analyze due to the limited availability of unified analytical tools. **Celtix™**, developed by **Tixomics™**, addresses this need as a fully integrated platform for end-to-end spatial analysis of highly multiplexed tissue imaging data.

Celtix™ integrates image quality control, cell segmentation, phenotyping, spatial statistics, and visualization within a single reproducible system. It scales efficiently from single slides to large multi-cohort studies while maintaining analytical consistency and computational efficiency.

Applied to a head and neck squamous cell carcinoma (**HNSCC**) cohort consisting of 5 responders and 5 non-responders, classified across four RECIST-defined clinical response groups (complete response [CR], partial response [PR], stable disease [SD], and progressive disease [PD]), and totaling over **8 million cells** profiled using a **47-antibody panel** generated with Akoya Biosciences' PhenoCycler-Fusion platform, Celtix™ enabled whole-slide, single-cell analysis of the tumor microenvironment (TME) and reproducible spatial profiling without reliance on external tools.

End-to-End Spatial Analysis Platform

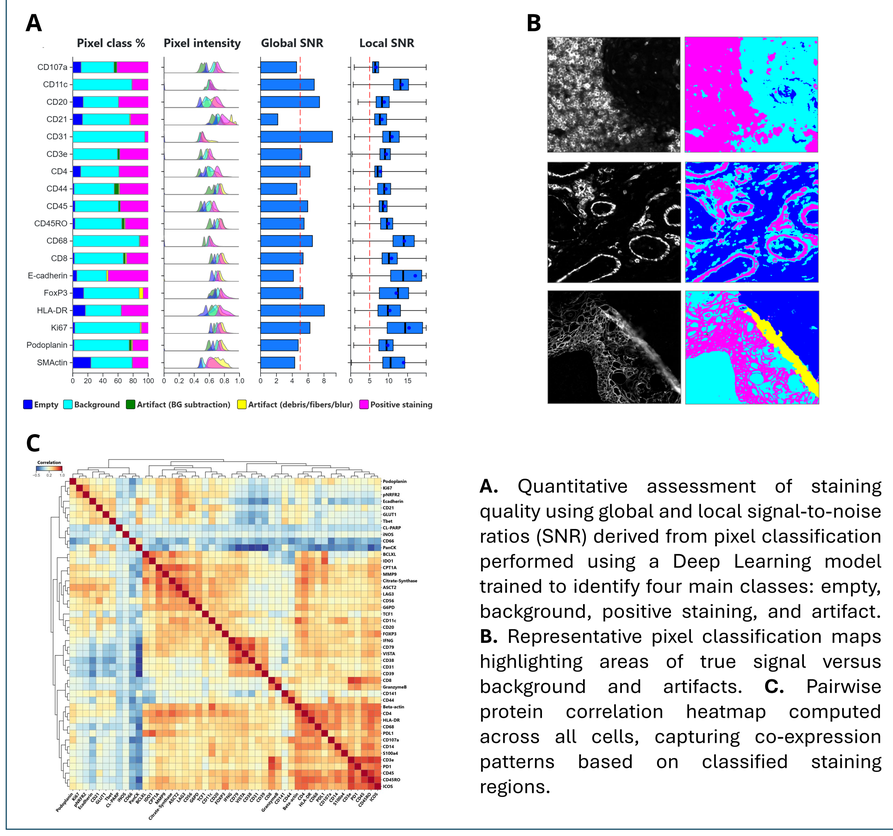
Celtix integrates data management and spatial analysis in a unified workflow. Users can organize datasets, structure studies, and collaborate through the Data, Study, and Group Managers.



Celtix Integrated Workflow and Management Framework

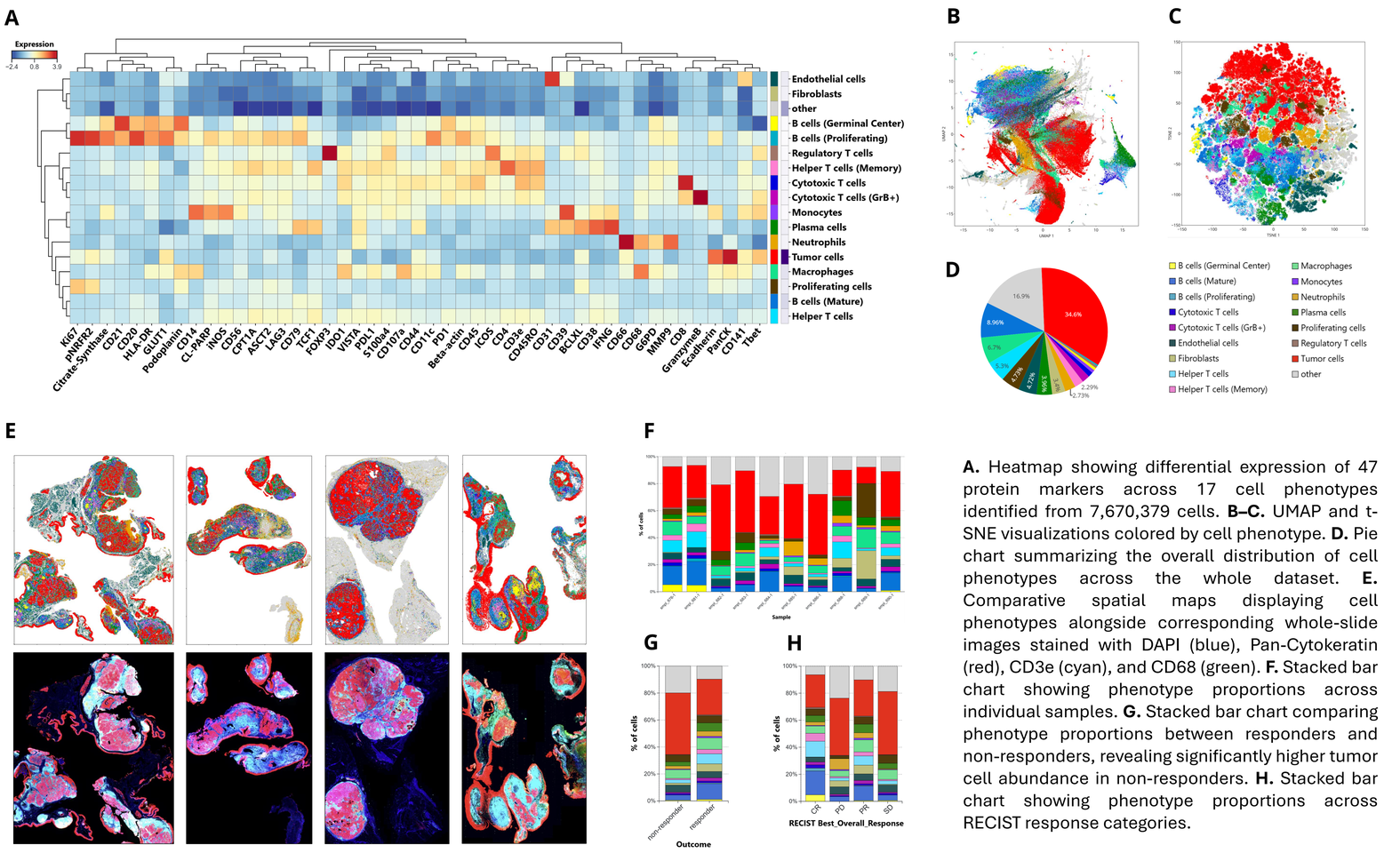
The analysis pipeline includes Study Design, Quality Control, Segmentation, Phenotyping, Structure Identification, and Spatial Analysis, automating tissue detection, quality assessment, cell segmentation, phenotyping, and spatial quantification within a scalable and reproducible framework.

Quality Control



A. Quantitative assessment of staining quality using global and local signal-to-noise ratios (SNR) derived from pixel classification performed using a Deep Learning model trained to identify four main classes: empty, background, positive staining, and artifact. **B.** Representative pixel classification maps highlighting areas of true signal versus background and artifacts. **C.** Pairwise protein correlation heatmap computed across all cells, capturing co-expression patterns based on classified staining regions.

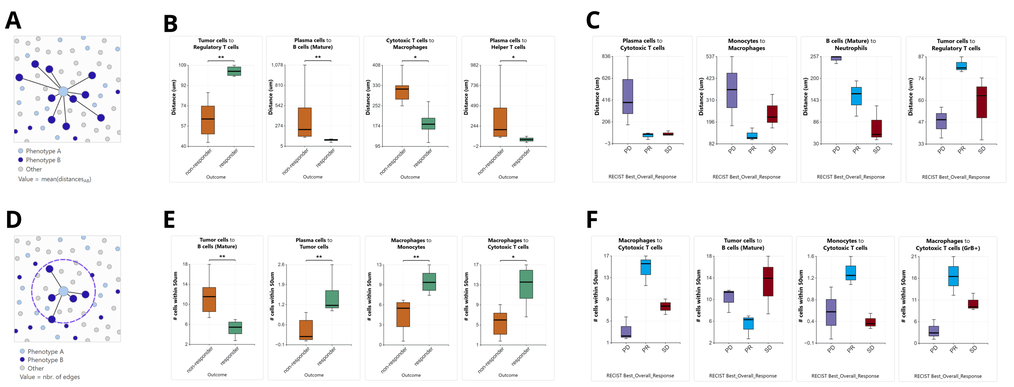
Cell Phenotyping



A. Heatmap showing differential expression of 47 protein markers across 17 cell phenotypes identified from 7,670,379 cells. **B–C.** UMAP and t-SNE visualizations colored by cell phenotype. **D.** Pie chart summarizing the overall distribution of cell phenotypes across the whole dataset. **E.** Comparative spatial maps displaying cell phenotypes alongside corresponding whole-slide images stained with DAPI (blue), Pan-Cytokeratin (red), CD3e (cyan), and CD68 (green). **F.** Stacked bar chart showing phenotype proportions across individual samples. **G.** Stacked bar chart comparing phenotype proportions between responders and non-responders, revealing significantly higher tumor cell abundance in non-responders. **H.** Stacked bar chart showing phenotype proportions across RECIST response categories.

Spatial Analysis

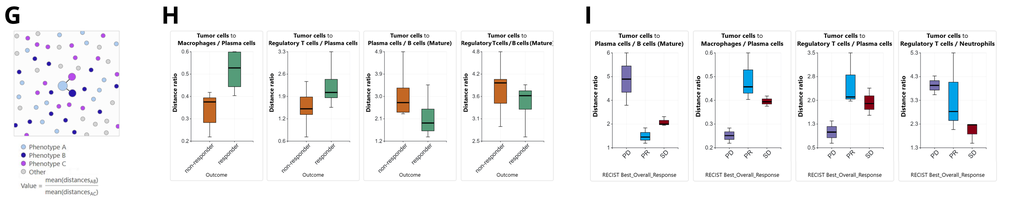
Spatial Proximity



A–C. *k*-Nearest Neighbor (*k*=10) analysis illustrating spatial proximity between cell phenotypes. **A** shows the schematic of *k*-NN distance computation, while **B–C** display mean intercellular distances between cell phenotypes across response categories, where shorter distances reflect higher spatial interactions.

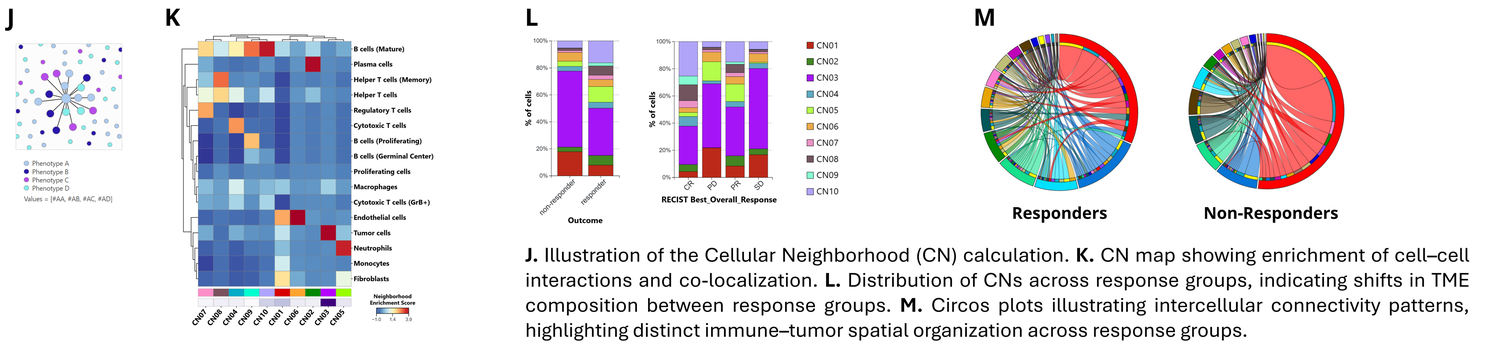
D–F. Neighborhood-based analysis quantifying local interactions within a 50 µm radius. **D** depicts the neighborhood model, and **E–F** show the number of neighboring cells per phenotype pair across response groups, where higher numbers reflect higher spatial interactions.

Spatial Score



G–I. Spatial score analysis measuring relative proximity ratios between tumor and immune cells. **G** illustrates the scoring principle, and **H–I** show spatial score distributions revealing distinct immune–tumor organization patterns linked to treatment response.

Cellular Neighborhood



J. Illustration of the Cellular Neighborhood (CN) calculation. **K.** CN map showing enrichment of cell–cell interactions and co-localization. **L.** Distribution of CNs across response groups, indicating shifts in TME composition between response groups. **M.** Circos plots illustrating intercellular connectivity patterns, highlighting distinct immune–tumor spatial organization across response groups.

Conclusion

Celtix provides a scalable and comprehensive solution for spatial proteomic analysis, supporting reliable investigation of tissue architecture and tumor immune interactions. By integrating all analytical steps into a single framework, it ensures reproducibility and consistency across large datasets. Celtix establishes a solid foundation for biomarker discovery and comparative studies in spatial biology.

Contact: bbencheikh@tixomics.com