Spatial Proteomics Meets AI: A Path to the Clinic

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Introduction

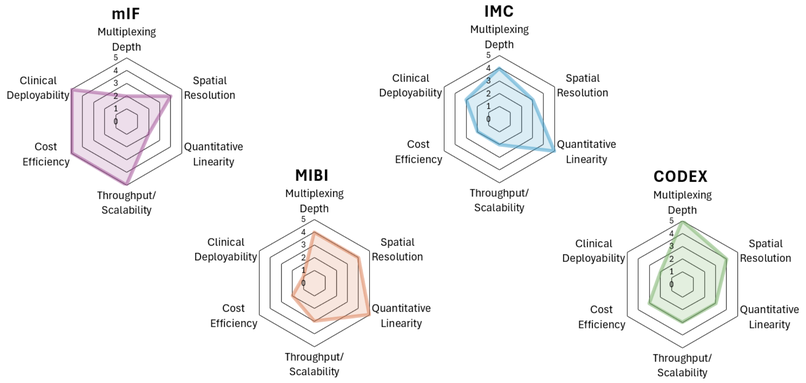
- **Problem:** The tumour microenvironment (TME) is spatially complex and poorly captured by low-plex methods
- **Opportunity:** High-plex spatial proteomics reveals single-cell biology with spatial context
- **Challenge:** Clinical translation requires reducing complexity into actionable biomarkers using artificial intelligence (AI)

Comparative Analysis

- To determine the most viable path to the clinic, four leading spatial proteomics technologies were evaluated based on their technical maturity, quantitative robustness, and operational scalability:
- **Multiplexed Immunofluorescence (mIF):** Low-plex, fast, and cost-effective fluorescence imaging suitable for routine pathology workflows
- **Imaging Mass Cytometry (IMC):** Laser ablation and metal-tagged antibodies enable highly multiplexed, quantitative single-cell imaging
- **Multiplexed Ion Beam Imaging (MIBI):** Ion beam-based imaging provides subcellular resolution with high plex capacity
- **Co-Detection by Indexing (CODEX):** Iterative imaging of DNA-barcoded antibodies enables very high-plex spatial profiling

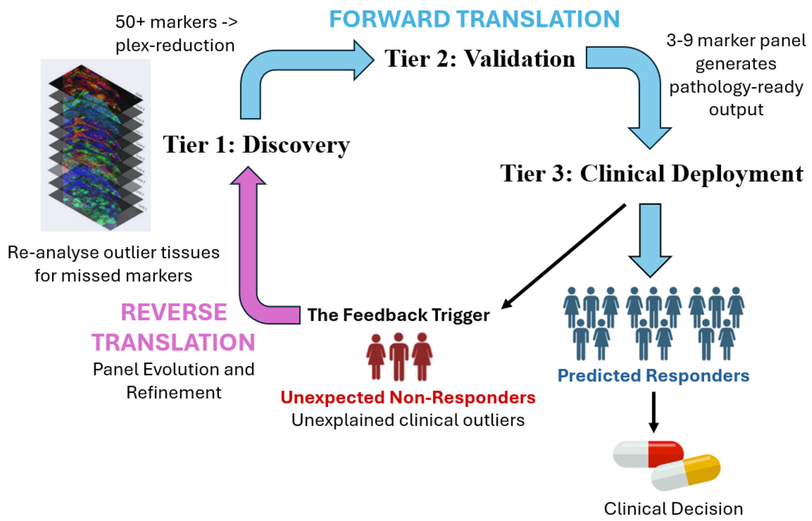
	mIF	IMC	MIBI	CODEX
Plex Capacity	5-12 markers	40+ markers	40+ markers	50-100+ markers
Spatial Resolution	~200-300 nm	~1 µm	~ 260 nm	~250-300 nm
Throughput	Fast	Very slow	Slow	Slow
Cost (Per Sample)	Low	Medium	Medium	Medium

- High-plex platforms provide deep spatial insight but slow and costly
- Low-plex platforms offer speed and clinical scalability



Three-Tiered Path to the Clinic

- Spatial biology is complex: clinical decisions require simplicity and reproducibility
- An original three-tiered framework aligns specific technological capabilities with successive stages of biomarker development



Tier 1: Discovery (CODEX/MIBI)
- **Goal:** Unbiased spatial mapping
- **Approach:** High-plex platforms identify thousands of data points, which are then processed through AI-driven plex reduction
- **Output:** Identification of distilled signature (3-9 markers) that captures essential spatial interactions

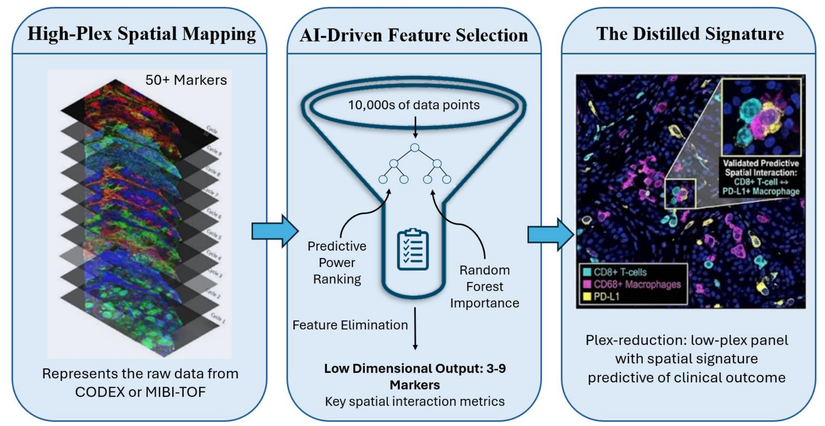
Tier 2: Validation (IMC)
- **Goal:** Test reproducibility across large, heterogenous cohorts

Tier 3: Clinical Deployment (mIF)
- **Goal:** Implementation within routine digital pathology workflows
- **Approach:** Transition the distilled panels to cost-effective, high-throughput platforms like mIF
- **Output:** Pathology-ready spatial scores for treatment decisions

Reverse Translation
- Clinical discrepancies trigger return to high-plex discovery to refine and evolve biomarker panels
- This ensures that clinical panels remain biologically relevant as tumour evolve and new resistance mechanisms emerge

Artificial Intelligence (AI)

- AI reduces >50 markers and complex spatial data into minimal, predictive biomarker panels using feature selection and machine learning
- These reduced panels identify validated spatial interactions that serve as refined biomarkers for therapy response



Future Directions

- Combining spatial proteomics with transcriptomics is essential to distinguish active immune engagement from phenotypic mimicry
- Generative AI prediction of protein expression from routine Haematoxylin & Eosin (H&E) images
- Clinical adoption requires community-wide benchmarking and black-box AI interpretability to ensure analytical validity
- Discovery-stage technologies must reach whole-slide imaging speeds and lower costs to reflect diverse patient demographics

References

[1] Angelo, M., Bendall, S.C., Finck, R., Hale, M.B., Hitzman, C., Borowsky, A.D., Levenson, R.M., Lowe, J.B., Liu, S.D., Zhao, S., Natkunam, Y. and Nolan, G.P. 2014. Multiplexed ion beam imaging of human breast tumors. *Nature medicine*. **20**(4), pp.436–442.

[2] Cohen, R., Lee-Pullen, T., Miller, T.J., Meehan, K., Fuller, K. and McCoy, M.J. 2023. Optimising multiplex immunofluorescence staining for characterising the tumour immune micro-environment. *Methods* (San Diego, Calif.). **219**, pp.48–57.

[3] Giesen, C., Wang, H.A.O., Schapiro, D., Zivanovic, N., Jacobs, A., Hattendorf, B., Schüffler, P.J., Grolimund, D., Buhmann, J.M., Brandt, S., Varga, Z., Wild, P.J., Günther, D. and Bodenmiller, B. 2014. Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry. *Nature methods.* **11**(4), pp.417–422.

[4] Goltsev, Y., Samusik, N., Kennedy-Darling, J., Bhate, S., Hale, M., Vazquez, G., Black, S. and Nolan, G.P. 2018. Deep profiling of mouse splenic architecture with CODEX multiplexed imaging. *Cell.* **174**(4), pp.968-981.e15.

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