

High-multiplex imaging workflow comparison

Technology	Prep	Stain	Acquire	Image	Analysis
Imaging Mass Cytometry™ (IMC™)	Uses metal-tagged Ab on tissue sections and cells on a standard microscope slide .	Stain with standard immunohistochemistry (IHC) protocol.	Manually pre-select region of interest (ROI) coupled to mass cytometry.	Signal extraction of n markers—single-scan acquisition at single-cell resolution.	Real-time quantitative IMC data is collected in a small single file that can be used to immediately generate publication-quality images.
Digital spatial profiling (DSP)	Uses oligo-conjugated Ab/RNA probes. Tissues are sectioned on a standard microscope slide .	Incubate tissue with fluorescent markers, antibodies and RNA probes tagged with photocleavable barcodes.	UV-cleave oligos on manually pre-selected ROI, aspirate, deposit in 96-well plate, hybridize for counting or purify for sequencing.	Ideally suited to investigating larger 200–400 μm ROIs as a complement to imaging-based methods. ^{2,3}	Digital counting by nCounter®/sequencing, ROI counts layered onto previously stained reference image.
CODEX®	Uses Ab-tagged oligos hybridized to complementary oligo-linked fluorophores. Tissues are sectioned onto a glass coverslip .	Stain with standard immunofluorescence (IF) protocol.	Perform fluorescent probe exposure, image, cleave to remove fluorophores and prep for next cycle.	Visualize selected fluorophores in each cycle to reconstruct digital image from all cycles.	Stitching and alignment⁴ of multiple images/tile areas collected from each cycle results in a large file size .
			Repeat for n cycles depending on number of parameter sets needed.		
MIBI™-TOF	Uses metal-tagged Ab and tissue sectioned on specialized gold slide .	Stain with standard IHC protocol.	Manually pre-select ROI coupled to mass spectrometry. ¹	Composite image of n markers at single-cell resolution.	Post-processing including alignment and MIBI-specific background removal before denoising .

The workflows for each approach vary from slide prep to data acquisition and analysis, including the need for specialized equipment and step repetition. IMC maintains a simple workflow while providing a comprehensive dataset at the single-cell level in a single scan. Published references validate the use of IHC or quantitative IF with IMC⁵⁻⁸. Green and red text highlights the differences between workflows. Red sections indicate the need to repeat steps. Note that other cyclic IF protocols on alternative platforms exist that include variations to the CODEX workflow.

Workflow impact on ease of panel design:

- Order and assignment of fluorescent-tagged antibodies used with CODEX must be well-planned to maximize signal strength with variable marker expression.
- Antibody options can also be limited, particularly with digital spatial profiling, where panels are fixed, and customization can be costly.
- Metal-tagged antibodies used for IMC and MIBI-TOF provide more flexibility to better customize panels with markers that can be easily substituted as experimental needs change.
- To simplify IMC, a selection of pre-designed panels can be used with or without additional markers.

References

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