

## **Mapping the spatial heterogeneity of the tumor microenvironment by hyperplex immunofluorescence with COMET™**

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Cancer cells and the tumor microenvironment (TME) surrounding them co-evolve with time, leading to spatial and temporal heterogeneity. The complexity of such crosstalk is also responsible for inter-patient heterogeneity and different response to therapy. For the advancement of tumor treatment, therefore, the need for personalized medicine has emerged, which in turn requires a precise molecular profiling at the individual level. By means of new spatial proteomic approaches, a detailed mapping of the TME has been made possible, deciphering complex tissue samples at the single cell level.

The COMET™ platform is a microfluidic-based instrument that allows a fully automated sequential immunofluorescence protocol, including staining and imaging of tissue samples. Through its microfluidic technology, it allows the rapid detection of up to 40 antigens on a single slide, therefore maximizing the information that can be retrieved per sample.

Here, we show the development of hyperplex immuno-oncology panels with COMET™. Markers were verified on tumor microarrays and positive control tissues like lymphoid organs, on which we demonstrated the possibility to perform a 40-plex staining with COMET™. Further analysis on frozen sections of human tumors showed that hyperplex panels can be run on multiple tissue types, displaying COMET™ value for the comprehension of the TME.