**Systems pathology by multiplexed immunohistochemistry and whole slide digital image analysis**

* Multiplexed marker detection to better comprehend the intricate disease processes
* But no systems exist that permit multiplexed IHC (mIHC) with high-resolution whole-slide tissue imaging and analysis while offering practicable throughput for daily use.
* Combining fluorescent and chromogenic staining, automated whole-slide imaging, and integrated whole-slide image analysis, enables the automatic quantification and classification of hundreds of thousands of cells in situ in formalin-fixed paraffin-embedded tissues, as well as the simultaneous detection of six protein markers and nuclei.
* In the first proof-of-concept, we identified immune cells in human prostate cancer at the cell-level resolution (n=128,894 cells) and analysed T cell subpopulations in several tumour compartments (epithelium vs. stroma).
* We showed automatic classification of epithelial cell populations (n=83,558) and glands (benign) in the second proof-of-concept.
* This is followed by a visual evaluation of antibody reactivity. Nevertheless, because the analysis of numerous markers is done on successive sections, it is unable to determine the co-localization of markers at the level of a single cell, which severely restricts the ability to classify cells accurately when several markers must be found (e.g. diferent Understanding the spatial cellular composition and tissue heterogeneity is crucial, particularly in cancer, where cell subpopulations and the tumour microenvironment offer insights on the biology and clinical course of the disease.
* advancement of the illness. Immunohistochemistry (IHC) on thin sections of formalin-fixed paraffin-embedded (FFPE) tissue is the standard procedure for detecting proteins in sit subtypes of immune cells). Tumours should be analysed in order to better comprehend the pathological processes, provide more precise prognostics, and patient stratification for treatments.
* We used whole-slide analyses of immune cells (Fig. 1) and prostate epithelial cells (Fig. 2) in human prostate cancer (PCa) samples as a proof-of-concept for the mIHC technique. This approach makes use of a combination.
* for the simultaneous detection of six proteins and nuclei in formalin-fixed tissue sections using fluorescent and chromogenic tissue labelling.