CCBIO Opinion

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THE ADVANCEMENT OF SPATIAL MAPPING TECHNIQUES – POSSIBILITIES AND CHALLENGES

The single cell

It was a technical and biological revolution, starting with the introduction of single cell sequencing techniques, that led to the description and characterization of novel cellular subtypes and cellular activation states. Consequently, researchers have gained a more detailed understanding of the cellular processes in health and disease. However, with the spatial information of the tissue context being lost during the enzymatic cell isolation processes, the relationship between cellular neighborhoods remains hidden, which still represents a key drawback of single cell sequencing methods.

Traditionally, pathology approaches such as immunohistochemistry or RNA *in situ* hybridization, have been standard tools to detect the spatial distribution of a defined target of interest within the tissue context. However, in light of the increasing amount of novel single cell data, these traditional approaches, with their limited ability to upscale the target number, have become insufficient to visualize complex contextual phenotypes.

Location matters – cellular ecosystems

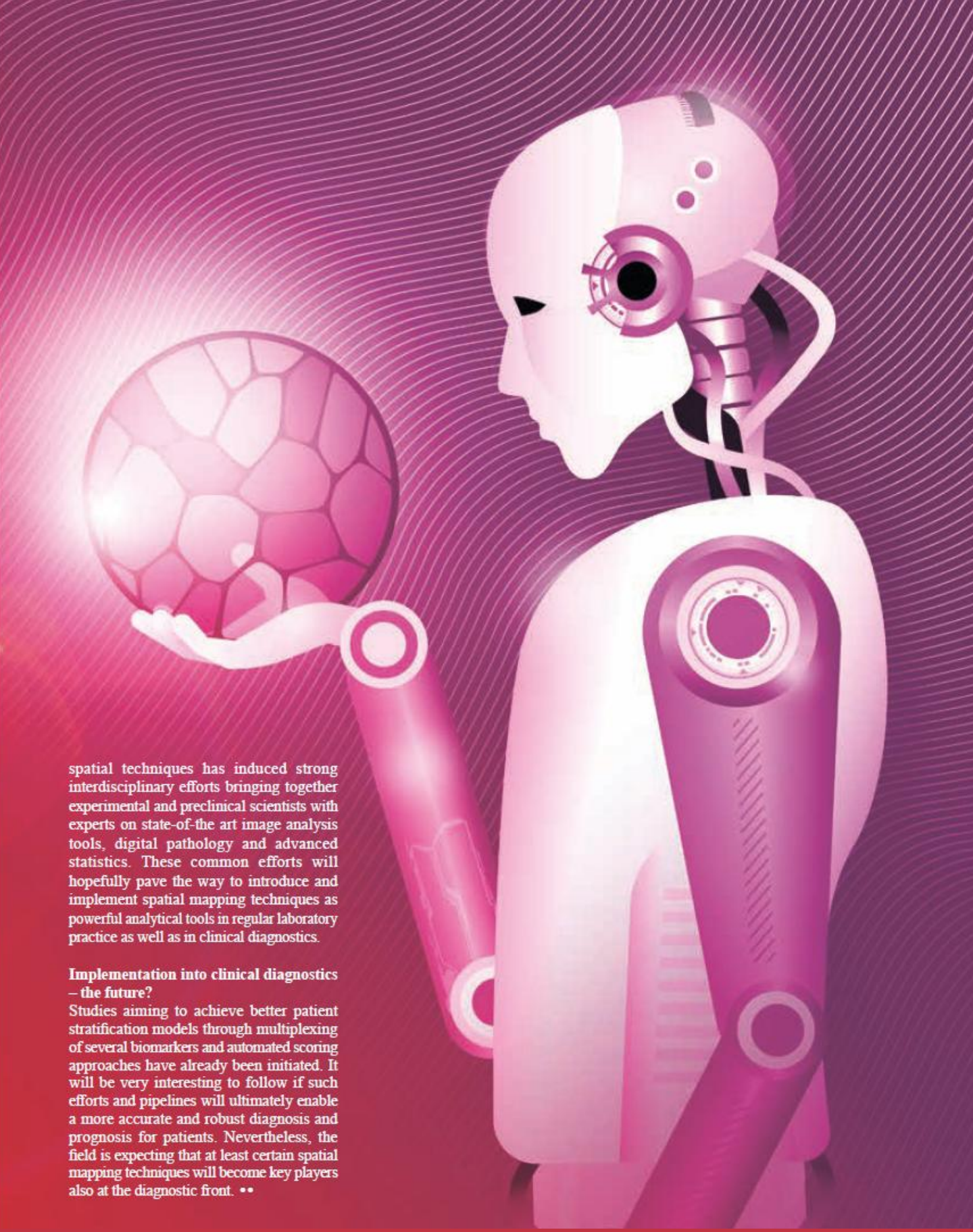
Pathologists, who work with the analysis of tissue sections on a daily basis, have always pointed out the importance of the spatial context. This information is more than just defining the anatomic location of a single cell. Spatial context is the indispensable information needed in order to fully understand cellular phenotypes, their plasticity, their interactions and, ultimately, their functionalities. This concept of cellular ecosystems, where genomic and microenvironmental interactions in concert determine the phenotype of a cell, had been postulated based on previous histological observations, but have received fresh and redefined attention with the new analytical possibilities opened by the accelerating development of highly multiplexed spatial mapping techniques.

Spatially mapping approaches currently cover a range from multiplexed protein detection (e.g. CODEX, Hyperion imaging mass cytometry) over RNA mapping (targeted: MERFISH *in situ* sequencing; untargeted: FISSEQ) to NGS based spatial transcriptomics (spatial transcriptomics); and some arising tools which combine both, RNA and protein detection. The high degree of multiplexing is achieved through different strategies including amongst others heavy-metal tagging of antibodies, or bar-code labeled probes, or iterative imaging cycles. Spatial mapping adds a deep informational layer to classical histology, revealing the expression of dozens of genes or proteins in one and the same tissue section, ideally at a single-cell level or even with subcellular resolution. These quantitative spatial insights allow us to gather new perspectives on disease progression or developmental processes. It is thus very understandable that researchers from almost any biological and medical field have been waiting for this technological advancement for years.

Challenges and possibilities ahead

Nevertheless, the overall excitement of the new spatial analysis tools is slightly shadowed by some technical challenges that still need to be overcome to make these approaches robust experimental tools for a laboratory day-to-day basis. Technical obstacles such as impaired sensitivity and/or specificity, low cellular resolution, and low sample throughput, often make spatial mapping approaches laborious to establish and use. Many of the spatial techniques are based on a fluorescent read-out and thus are easily impacted by tissue autofluorescence as well as staining artefacts. Consequently, each generated image and marker needs to be carefully reviewed and evaluated by pathologists. The implementation of spatial mapping approaches in the experimental design therefore requires careful considerations, weighting the *pros and cons* of one approach against the other. Shall one go for protein or RNA mapping, which sample type can be analyzed, how many markers are required, and which sensitivity and sample throughput is needed in order to obtain data that will answer the hypotheses or allow meaningful exploratory analyses. These decisions should always be reflected within the actual scope of the study. Finally, especially for young PIs, financial aspects come into the picture, as spatial experiments are still within the higher cost range.

Another, though highly exciting challenge, concerns data analysis and especially the interpretation of high-dimensional data. New computational algorithms are necessary to handle, relate and statistically analyze the compiled spatial data. Many analytical tools start with the problematic process of the accurate segmentation of single cells in the tissue context, which actually is the prerequisite to successfully interpret spatial phenotypes. Alternative approaches using pixel classification, AI or probabilistic cell phenotyping are increasingly considered and becoming established. In other words, the availability of new and highly multiplexed



spatial techniques has induced strong interdisciplinary efforts bringing together experimental and preclinical scientists with experts on state-of-the art image analysis tools, digital pathology and advanced statistics. These common efforts will hopefully pave the way to introduce and implement spatial mapping techniques as powerful analytical tools in regular laboratory practice as well as in clinical diagnostics.

Implementation into clinical diagnostics – the future?

Studies aiming to achieve better patient stratification models through multiplexing of several biomarkers and automated scoring approaches have already been initiated. It will be very interesting to follow if such efforts and pipelines will ultimately enable a more accurate and robust diagnosis and prognosis for patients. Nevertheless, the field is expecting that at least certain spatial mapping techniques will become key players also at the diagnostic front. ••