Tools of the trade

CODEX multiplexed tissue imaging

Co-detection by indexing (CODEX) is a recently developed and commercially available (Akoya Biosciences) technique of highly multiplexed tissue imaging — visualizing up to 60 markers at the single-cell level[1,2]. It belongs to a set of re-iterative fluorescent tissue imaging methods. Yet what sets CODEX apart from other comparable approaches, such as cyclic immunofluorescence, multiplex immunohistochemistry and Cell DIVE, is that in any given CODEX staining all the antibodies can be applied to the tissue sample at the same time. This vastly reduces the time needed for staining and aids in epitope preservation, which is hard to maintain during cycles of fluorophore bleaching or antibody stripping. This simultaneous staining is achieved through the use of antibodies with covalently conjugated DNA oligonucleotides (which are later revealed by fluorescent detector oligonucleotides) as primary antibody barcodes.

The CODEX procedure involves two major steps[2] (Fig. 1). First, the tissue sample is stained with DNA-barcoded antibodies. This is a well-described but complicated manual multistep procedure that can take a couple of days. Recently, this process has been automated using the Parhelia Omni-Stainer system, which aims to reduce the cost and supports the standardization of data collection across sites and researchers. The second step involves automated multicycle (three at a time) imaging of barcodes using the detector oligos conjugated to fluorophores. The fluorescent detector oligos are removed by duplex-melting washes between imaging cycles. The consequent analysis involves image preprocessing, segmentation, cell calling and eventually spatial feature extraction.

The key benefits of CODEX come from the use of standard four-colour fluorescent microscopy for imaging. This makes it feasible to image large microarrays of patient biopsy samples using mid-resolution air objectives and hence is high throughput. CODEX can also be run at higher resolution with immersion objectives — for example, to image the smaller cells of mouse tissues or the subcellular proteome at nanometre scales when combined with super-resolution techniques such as stochastic optical reconstruction microscopy (STORM). The standard version of CODEX produces around 1 terabyte of data per 1 square centimetre of tissue. These data are three-dimensional, which is crucial for RNA quantification (distributed as isolated dots of staining across the many planes of the cellular volume) together with protein markers. However, the volumetric data can be difficult and expensive to store and manage. With the development of neural net-assisted segmentation algorithms, recent versions of CODEX allow single plane images, which are fast to acquire and segment and do not take much space or expense to store.

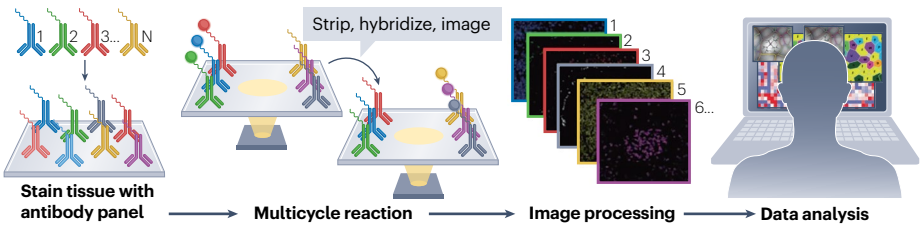

Fig. 1 | The CODEX procedure. Reproduced from ref. 3, Springer Nature Limited.

CODEX enables a deep view into tissue architecture and cellular organization. Several studies have higlighted how CODEX data support our understanding of the role of the cellular microenvironment in disease. The original CODEX paper examined unique features of splenic architecture in mice with autoimmune disease, and showed that the local niche effects levels of key functional proteins in immune cells[1]. This technology is currently being used to generate 3D spatial single-cell maps of healthy and cancerous tissues. CODEX has since been used to show, for example, that infiltration of specific microenvironments by activated immune cells informs prognosis of patients with colorectal cancer[4], and differential positioning of activated immune cells between tumour cells and regulatory T cells defines the responsiveness of patients with cancer to immunotherapy. The same authors have used CODEX to show that cellular neighbourhoods rather than individual cells define the basic functional units of tissues. They found that co-option of tumour cell neighbourhoods into neighbourhoods enriched for T cells, macrophages and vasculature markedly changes the level of immune resilience to colorectal cancer[4].

The future of explorative multiplexed data is likely to come from integrative studies in which deep single-cell RNA sequencing datasets are merged with spatial data to reveal the genes implicated in cell-to-cell communication and spatial tissue organization. At present, data preprocessing and analysis, rather than data acquisition, are the most time-consuming and expensive aspects, thus future efforts should be directed towards developing analytical tools that are accessible to academic trainees and non-computational biologists. From a clinical perspective, it is hoped that smaller sized panels of selected biomarkers that emerge from spatial studies may be validated for use in disease diagnosis.

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Competing interests

Y.G. and G.N. are among the founders and shareholders of Parhelia Biosciences and Akoya Biosciences.