Peer Review File

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Reviewer Comments

Comment 1: The paper indeed mentioned a broad range of imaging techniques, such as CODEX, IMC, mIF, etc. However, a significant number of key publications failed to be properly cited. These include but not limited to:
(1) Goltsev, Yury, et al. "Deep profiling of mouse splenic architecture with CODEX multiplexed imaging." Cell 174.4 (2018): 968-981. [For CODEX]
(2) Black, Sarah, et al. "CODEX multiplexed tissue imaging with DNA-conjugated antibodies." Nature protocols 16.8 (2021): 3802-3835. [For CODEX]
(3) Chang, Qing, et al. "Imaging mass cytometry." Cytometry part A 91.2 (2017): 160-169. [For IMC]
(4) Lin, Jia-Ren, et al. "Highly multiplexed immunofluorescence imaging of human tissues and tumors using t-CyCIF and conventional optical microscopes." elife 7 (2018). [For CyCIF]
(5) Keren, Leeat, et al. "MIBI-TOF: A multiplexed imaging platform relates cellular phenotypes and tissue structure." Science advances 5.10 (2019): eaax5851. [For MIBI]
Reply 1: T Thank you for this comment. Indeed, we did not provide references specifically describing a given method, hoping that the official product brochures will be enough to give the readers the technology/product name and basic characteristics so that they (the readers) can use that info to search for more details about technology of interest. Following your suggestions, we introduced Goltsev et al and Black et al for CODEX (refs. 3 and 4 in the R1 version of the manuscript), Chang et al for image mass cytometry (ref. 5 in R1), and Keren et al for MIBI-TOF (ref. 6 in R1). Lin JR et al are introduced for CyCIF in Table 1.

Comment 2: References for analyses using single-cell spatial data that derived from aforementioned techniques are almost completely missing. These include but not limited to:
(1) Greenwald, Noah F., et al. "Whole-cell segmentation of tissue images with human-level performance using large-scale data annotation and deep learning." Nature biotechnology 40.4 (2022): 555-565.
(2) Schürch, Christian M., et al. "Coordinated cellular neighborhoods orchestrate antitumoral immunity at the colorectal cancer invasive front." Cell 182.5 (2020): 1341-1359.
(3) Moldoveanu, Dan, et al. "Spatially mapping the immune landscape of melanoma using imaging mass cytometry." Science Immunology 7.70 (2022): eabi5072.
(4) Mi, Haoyang, et al. "Digital pathology analysis quantifies spatial heterogeneity of CD3, CD4, CD8, CD20, and FoxP3 immune markers in triple-negative breast cancer." Frontiers in physiology 11 (2020): 583333.
(5) Jackson, Hartland W., et al. "The single-cell pathology landscape of breast cancer." Nature 578.7796 (2020): 615-620.

(6) Mi, Haoyang, et al. "Predictive models of response to neoadjuvant chemotherapy in muscle-invasive bladder cancer using nuclear morphology and tissue architecture." Cell Reports Medicine 2.9 (2021).
(7) Berry, Sneha, et al. "Analysis of multispectral imaging with the AstroPath platform informs efficacy of PD-1 blockade." Science 372.6547 (2021): eaba2609.
(8) Mi, Haoyang, et al. "Quantitative spatial profiling of immune populations in pancreatic ductal adenocarcinoma reveals tumor microenvironment heterogeneity and prognostic biomarkers." Cancer research 82.23 (2022): 4359-4372.
(9) Mi, Haoyang, et al. "Multi-scale spatial analysis of the tumor microenvironment reveals features of cabozantinib and nivolumab efficacy in hepatocellular carcinoma." Frontiers in immunology 13 (2022): 892250.
(10) Nakhli, Ramin, et al. "Sparse Multi-Modal Graph Transformer With Shared-Context Processing for Representation Learning of Giga-Pixel Images." Proceedings of the IEEE/CVF Conference on Computer Vision and Pattern Recognition. 2023.

Reply 2: We do agree with you that specific references about the use of spatial tissue analysis technologies on single cells were missing in the initial submission. It was done intentionally as many of the mentioned technologies can be used on single cells (as indicated in Table 1, column 4), so we thought that elaborating on that subject would be somewhat “duplicating”. Furthermore, we did read the papers you suggested to cite, however can not agree with you that all of them speak about the spatial technologies used for single-cell analysis. In our humble opinion, only Moldoveanu et al, and Jackson HW et al treat that matter, whereas other articles mostly present the results of cell group/tissue areas analysis by spatial technologies. However, we do acknowledge that the readers should be provided with a few review articles in which the use of the mentioned technologies on single cells will be described and critically evaluated – that’s why we introduced three very recent and detailed reviews (refs. 34, 35, 36) in the lowest line of Table 1. Hope this solution will be acceptable to you.