Peer Review Report

Review Report on Advancements in Single-Cell RNA Sequencing and Spatial Transcriptomics: Transforming Biomedical Research

Review, Acta Biochim. Pol.

Reviewer: Arkadiusz Piotrowski
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EVALUATION

Q 1 Please summarize the main theme of the review.

The main theme of the review by Desta and Birhanu are advancements of single-cell RNA sequencing and spatial transcriptomics technologies, which have transformed the ability to analyze transcriptomic information at the individual cell level. The review emphasizes the role of scRNA-seq and spatial transcriptomics in understanding cellular heterogeneity and its applications across various biomedical fields, including cancer research, neuroscience, and immunology. The manuscript aims to provide a comprehensive review of these technologies, their applications, challenges, and future directions, ultimately contributing to the fields of genomics, bioinformatics, and clinical research.

Q 2 Please highlight the limitations and strengths.

Strengths:
1) The manuscript is generally well-organized and divided into clearly defined sections, although individual paragraphs in these sections need some connecting phrases to improve flow (see my detailed comments below).
2) The included tables and figures support the content and aid readers in understanding complex information, although they could benefit from some improvements – see my detailed comments below.

Limitations:
1) Overly broad scope results in covering too many aspects superficially, particularly in data analysis and application sections.
2) Imbalance between presentation of scRNA-seq and spatial transcriptomics.
3) The manuscript's sections, especially early on, contain many short, disconnected paragraphs that impair the flow.
4) Some statements are repeated unnecessarily, such as general advantages of scRNA-seq over bulk methods.

Q 3 Does the review include a balanced, comprehensive and critical view of the research area?

While the manuscript covers a broad scope and includes relevant technical insights, the overly extensive focus results in superficial descriptions, particularly in data analysis and application sections. Additionally, there is a noticeable imbalance between scRNA-seq and spatial transcriptomics description.

Check List

Q 4 Is the English language of sufficient quality?

Yes.

Q 5 Is the quality of the figure(s) and/or table(s) satisfactory?

No.

Q 6 Does this manuscript refer only to published data? (unpublished or original data is not allowed for this article type)

Yes.

Q 7 Does the manuscript cover the topic in an objective and analytical manner

No.

Q 8 Does the reference list cover the relevant literature adequately and in an unbiased manner?

Yes.

Q 9 Does the manuscript include recent developments?

Yes.

Q 10 Does the review add new insights to the scholarly literature with respect to previously published reviews?

Yes.

Q 11 Please provide your detailed review report to the editor and authors (including any comments on the Check List)

The manuscript is generally well-organized and divided into clearly defined sections. However, the text within these sections, particularly before Section 3.2, is overly fragmented, with many short paragraphs lacking clear connections. This fragmentation, which persists throughout the manuscript, makes it difficult to follow. To improve readability, the authors should work on refining the manuscript's structure to enhance flow.
The manuscript also covers an overly broad scope. This broad approach results in certain aspects, such as data analysis, being described only superficially. Narrowing the focus to single-cell and spatial transcriptomics—along with a comparison of available techniques and their respective advantages and disadvantages—would improve depth. Similarly, the section on “single-cell sequencing applications in biomedical research” attempts to address too many applications and, as a result, lacks depth. Focusing on select applications, such as in cancer research and immunology, or omitting this section entirely to strengthen other parts of the manuscript, would be advisable.
Some redundancies in the text also reduce clarity. For example, general statements on the advantages of scRNA-seq over bulk methods are repeated unnecessarily. Additionally, while describing the limitations of bulk RNA-seq compared to scRNA-seq, it would be beneficial to mention that deconvolution analysis can partially alleviate these limitations.

Certain passages lack clarity or precision; e.g. lines 100–102 should be revised for better comprehension. Additionally, the “Data Preprocessing” section would logically precede the discussion of downstream analyses, such as normalization.
In terms of figures and tables, Figure 1 should also indicate the inherent biases of scRNA-seq methods, such as limited transcript representation and 3’- or 5’-end biases in some techniques. This would complement the description in lines 179–185. When addressing the challenges of full-length transcriptomic sequencing methods (lines 186–195), it would also be helpful to mention their main advantage: the ability to combine transcriptomic and genotyping information, especially for studies exploring the transcriptomic effects of pathogenic genetic variants.
Further improvements in the tables and figures would also aid readers. For instance, Table 1 points 3 and 4 require more precise wording. Additionally, a figure (e.g., a separate panel of Figure 2 or a flowchart to guide selection based on intended applications – in supplement to text in lines no. 176–178) or an expanded version of Table 2 comparing different types of scRNA-seq methods would be useful. More detailed mechanistic explanations of lesser-known techniques would also benefit readers.
Please modify line 316 to read: “Methods implemented in Seurat…” and review lines 322–326 to improve consistency.
There is a noticeable imbalance between the descriptions of scRNA-seq and spatial transcriptomics techniques, with spatial transcriptomics receiving a much briefer treatment. Furthermore, it should be noted that many available spatial transcriptomics techniques preserve spatial information at the expense of analytical resolution, meaning that these methods are not strictly single-cell, although close.

QUALITY ASSESSMENT

Q 12	Quality of generalization and summary	
Q 13	Significance to the field	
Q 14	Interest to a general audience	
Q 15	Quality of writing