General Classification of Spatial Profiling Technology

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Spatial profiling technologies fill the gap left by the loss of spatial information in traditional single-cell sequencing, showing great application prospects. After just a few years of quick development, spatial profiling technologies have made great progress in resolution and simplicity.

Keywords: spatial transcriptomics ; spatial multi-omics sequencing ; in situ capture and sequencing

1. Introduction

Traditional single-cell sequencing technologies rely on tissue dissociation in the acquisition of single cells, which abandons the spatial positional information of different types of cells. However, the growth and differentiation of cells are influenced by the environment in which they are located. Therefore, ignoring the spatial location information cannot accurately determine the microenvironment of the cells, which is not conducive to the subsequent analysis of cell function. The development of spatial omics technology has filled in the blank areas of cellular organization and interactions in omics analysis. Meanwhile, it has unique advantages in the analysis of specific gene expression and cell localization and can provide key information for the research of detailed molecular mechanisms.

Therefore, positioning cells by spatial omics technology can explore the differentiation trajectory and development of cell lineages [1][2], which may have great application prospects in developmental biology. At the same time, spatial profiling technology can directly profile the heterogeneity of gene expression in different tissue regions, making it suitable for studying the heterogeneity of malignant tumors [3][4][5]. The utilization of spatial omics technology can associate transcriptomes with morphology and physiology when determining cell types, thus constructing a more precise spatial cell subtype map. Therefore, spatial omics technology has many contributions to make in the study of brain neural circuits [6][7][8]. Additionally, spatial sequencing technology also has new applications in the fields of novel coronavirus infection immune response [9], viral infection [10], plant tissue research [11], and so on.

2. General Classification of Spatial Profiling Technology

In general, spatial resolution transcriptomics is mainly divided into two categories: One is an image-based method based on *in situ* hybridization, in which staining and barcoding RNA in its original position are the key points to achieve multiple detection. The second category is based on *in situ* capture and sequencing: the spatial information is marked by barcoded primers attached to a solid surface, and then the spatial information is retrieved by high-throughput sequencing. Image-based methods originating from single-molecule RNA *in situ* hybridization are mainly divided into fluorescence *in situ* hybridization and fluorescence *in situ* sequencing. Fluorescence *in situ* hybridization uses labeled fluorescent probes to determine RNA/DNA abundance in tissues or cells, and the first example of imaging a single RNA species can be attributed to single-molecule fluorescence *in situ* hybridization (smFISH) [12], which provides absolute quantification of the copy number and localization of RNA molecules in cells. However, due to the limitation of fluorescence signal overlap, it can only be used for imaging low-abundance RNA. Sequential fluorescence *in situ* hybridization (seqFISH) [13] is a multiplexed smFISH with tens of thousands of distinct transcripts detected. Through continuous rounds of hybridization, imaging, and probe stripping, a single transcript is detected multiple times, which improves the target detection throughput. The existing fluorescence *in situ* hybridization methods have advantages in spatial resolution and target detection throughput, but they are expensive and time-consuming due to the need to synthesize a large number of fluorescent probes and can only measure known target transcripts.

In contrast, fluorescence *in situ* sequencing methods use single-base-specific fluorescence hybridization and DNA ligation reactions to determine the sequences of known or unknown transcripts in tissues. The representative technology is *in situ* sequencing (ISS) [3], which provides a highly multiplexed *in situ* detection scheme for different types of RNA by color sequence coding multiple imaging, which is not limited by the number of spectral and microscopic detection channels. Although the ISS method achieved *in situ* sequencing of the transcripts for the first time, only the known sequences of the

target sites can be determined. Fluorescent *in situ* sequencing (FISSEQ) technology [14] uses random primers to generate complementary DNA (cDNA) and then circularizes the cDNA by single-stranded DNA cyclase. After magnifying the rolling circle amplification (RCA) signal, the transcript itself is sequenced to obtain the transcript sequence information, and the RNA expression at the transcriptome level can be located and analyzed simultaneously in a non-targeting manner. Image-based spatial transcriptomics technology theoretically has high spatial resolution, but due to factors such as optical crowding, there is only a low gene number detection throughput.

With the rapid development of *in situ* capture technology, high-throughput single-cell multi-omics sequencing technology, and increasingly sophisticated microfluidic manipulation technology, spatial omics sequencing methods have dramatically developed. It has the advantages of high throughput, high sensitivity, a large tissue area for analysis, and the ability for multimodal analysis, making it an important technique for single-cell spatial resolution analysis.

The application of spatial profiling technology is of particular concern. In theory, any tissue containing active mRNA or intact, well-fixed mRNA is suitable for spatial sequencing technology. However, due to the defects of existing methods, the application of spatial omics is limited. When choosing the tissues, try not to choose the degraded FFPE tissues or the fragile fresh frozen tissues. When placed on a slide or chip for analysis, the tissue section is easy to tear and deform. Therefore, rare and valuable tissue samples or tissues that need serial section sequencing may have technical operation problems. At the same time, low-resolution spatial barcoded chips will cause important information to be missing, especially when applying some porous tissue sections.

It can be seen that to expand the spatial sequencing application range, resolution and gene detection number are key points. To improve this, two directions can be taken: spatial barcoded array design and last-step single-cell sequencing. Barcoded oligo sequences in liquid have diffusion issues, which will hinder the tight arrangement of the barcoded array, thereby further reducing resolution. The use of solid-phase microspheres or microarray sample applicators may alleviate this problem. The high-sensitivity sequencing method is also another focus to improve the quality of spatial sequencing technology. By simultaneously improving the fabrication of the upstream spatial array and the downstream sequencing sensitivity, the application range will become wider and wider.

Lastly, spatial omics technology is widely desired; however, many researchers have a relatively high threshold to operate and obtain spatial sequencing information because of the complexity of the operation, leading to a huge demand for commercial spatial omics sequencing platforms. At present, mature commercial platforms are developed using early spatial sequencing technology with limited performance. Therefore, the commercialization of high-resolution advanced spatial sequencing technology is also one of the development directions for spatial sequencing technology.

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