VIEWPOINT

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Single-cell omics: Overview, analysis, and application in biomedical science

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Abstract
Single-cell sequencing methods provide the highest resolution insight into cellular heterogeneity. Owing to their rapid growth and decreasing cost, they are now widely accessible to scientists worldwide. Single-cell technologies enable analysis of a large number of cells, making them powerful tools to characterise rare cell types and refine our understanding of diverse cell states. Moreover, single-cell application in biomedical sciences helps to unravel mechanisms related to disease pathogenesis and outcome. In this Viewpoint, we briefly describe existing single-cell methods (genomics, transcriptomics, epigenomics, proteomics, and mulitomics), comment on available analysis tools, and give examples of method applications in the biomedical field.

KEYWORDS
bioinformatics, epigenomics, genomics, haematology, multiomics, proteomics, single-cell methods, transcriptomics

1 | INTRODUCTION

Human tissues are composed of heterogeneous cell populations that harbour different cell types. With the help of bulk sequencing, where individual cells cannot be distinguished from each other, many valuable insights into tissue dynamics have already been obtained. However, this approach only captures average properties of the population constituents, which often do not accurately portray the state of an individual cell.[1] In recent years, development of single-cell methods allowed us to identify the heterogeneity and cell-to-cell variations from a new perspective by providing a complete and unbiased analysis of each cell. The rising popularity of single-cell genomics was subsequently recognized as "Method of the Year in 2013," and most recently, the combination of methodologies, single-cell multiomics, was awarded "Method of the Year 2019."[2] The detailed

technical aspects of the numerous single-cell approaches have already been reviewed elsewhere,[3–6] and will, therefore, not be covered here. The aim of this Viewpoint article is to provide a summary of the various single-cell omic approaches, briefly cover analysis methods and present examples of their applications in biomedical sciences.

2 | SINGLE-CELL GENOMICS

Single-cell DNA sequencing (scDNA-seq) helps to unmask intercellular variation and heterogeneity at genomic level. Currently scDNA-seq methods allow to study single nucleotide variants, copy number variations and microsatellite variations.[7–9] Cancer biology is one of the research areas where scDNA-seq is widely applied. It can be used to trace the expansion of different clones and reconstruct cell lineages in the mosaic tissue of the tumour.[10,11] In addition, scDNA-seq has the power to characterise rare cell types (e.g., cancer stem cells) that would have been missed in conventional bulk analyses.[12] Despite the benefits, numerous challenges still exist that need to be addressed such as selection bias caused by the whole genome amplification step. As a single cell only holds two copies of genomic DNA with an approximate weight of 6 pg, a wide variety of amplification methods had to be developed to obtain enough material for library preparation. More recently, microfluidic systems were used to build libraries without preamplification steps by direct tagmentation of single-cell DNAs that reach a more uniform coverage and provide high-resolution single-cell copy-number profiles.[13] Other limiting factors include the throughput of cell isolation techniques, the occurrence of allele dropout events, loss of coverage uniformity, and false-positive, false-negative errors.[14,15]

3 | SINGLE-CELL TRANSCRIPTOMICS

Single-cell transcriptomics have developed rapidly ever since 2009, when the first single-cell transcriptome profile was described.[16] Cell populations with homogenous cell surface markers harbour cell-to-cell variations with a considerable impact on cell function.[17] This heterogeneity can be resolved using single-cell RNA sequencing (scRNA-seq). Currently available scRNA-seq technologies can be divided into two categories: (1) droplet-based (e.g., Drop-seq,[18] inDrop,[19] 10x Genomics,[20] Seq-well[21]) and (2) plate-based (e.g., STRT-seq,[22] Smart-Seq. 1–3.[23–25] All available methods are based on the conversion of RNA into complementary DNA followed by amplification steps to obtain sufficient amounts of DNA for sequencing. Plate-based approaches (e.g., Smart-seq) generate full-length transcripts that can detect lowly expressed genes, alternative splicing events and allele-specific expression. Droplet-based methods (e.g., 10x Genomics), on the other hand, can analyse a larger number of single cells, however, since they sequence only the 5′ or 3′ end of the transcript, no allele-specific expression or isoforms can be detected.[26]

Single-cell transcriptomics is the most mature among single-cell methods. However, it also harbours several limitations. The majority of the currently available protocols only focuses on the polyadenylated messenger RNA (mRNA) fraction, thus excluding microRNAs and other regulatory RNAs from the analysis. To circumvent that problem, new methods have been developed that capture the entire RNA content of a single cell.[27] Another problem stems from tissue processing that can lead to both a distorted reflection of the composition of the original tissue and altered gene expression patterns.[28,29] To avoid that, new protocols have been developed that aim to minimise gene expression artefacts related to tissue preprocessing.[30]

4 | SINGLE-CELL EPIGENOMICS

Epigenetics is a complex regulatory network that modulates chromatin structures and genome function via chemical modifications of DNA and histone proteins.[31,32] Currently available single-cell methods focus on the investigation of the methylation pattern and chromatin state.

4.1 | Methylation profile

The analysis of single-cell DNA methylation typically relies on methylation-sensitive restriction enzymes and bisulfite conversion. The quantitative information of bisulfite sequencing is considered the gold standard for genome-wide methylation analysis, but its application to single cells is hampered by DNA degradation, resulting in high dropout rates.[38] Single-cell bisulfite sequencing (scBS-seq) allows the detection of the 5mC methylation status at CpG sites, genomic regions where a cytosine nucleotide is followed by a guanine nucleotide.[39] The main limitation of this method is a relatively poor genome coverage of only up to 48% with high sequencing depth in a single cell. Due to the lack of genome-wide coverage, allele-specific differences in methylation are difficult to detect.[40] Alternative to scBS-seq is single-cell reduced representation bisulfite sequencing (scRRBSseq).[41,42] Compared with the scBS-seq technique, scRRBSseq covers fewer CpG sites, but it provides better coverage for CpG islands, which are likely to be the most informative elements for DNA methylation.[42]

4.2 | Chromatin state

Single-cell techniques can also assess chromatin accessibility by transposase-accessible chromatin sequencing (ATAC-seq).[33] Currently, both plate-based (interrogation of hundreds of cells) and droplet-based (interrogation of tens of thousands of cells) methods for scATAC-seq are available.[34,35] Additionally, DNA-protein interactions in single cells can be investigated via chromatin immunoprecipitation sequencing (ChIP-seq).[36] This method identifies binding sites of DNA-associated proteins. Recently also methods allowing for investigation of histone modifications at single-cell level are becoming available.[37] For example, single-cell Cleavage Under Targets and Tagmentation (scCUT&Tag) allows for profiling diverse chromatin components at single-cell resolution including histone acetylation.[37]

5 | SINGLE-CELL PROTEOMICS

Proteome analysis at single-cell level can provide additional information on state and function of a cell. However, analysing the protein content of a single cell is challenging, mainly due to the lack of methods for protein amplification and the added complexity of secondary and tertiary structures. In recent single-cell studies, cytometry approaches based on fluorescence-activated cell sorting and single-cell mass spectrometry (CyTOF) became available with medium throughput (~40–50 proteins). CyTOF has been used to analyze surface and intracellular proteins by using antibodies, conjugated to rare heavy metal isotopes. This method overcomes the spectra overlap issue characteristic for multicolor flow cytometry.[43]

6 | SINGLE-CELL MULTIOMICS

Ultimately, the combination of the aforementioned single-cell sequencing techniques has raised attention as it allows for parallel analysis of multiple molecular features within the same cell.[4] One type of methods in single-cell multiomic studies includes genome and transcriptome sequencing (G&T-seq) which allows for simultaneous interrogation of DNA and mRNA from a single cell to dissect the effect of genomic variation on gene expression.[44] Another type of multiomic methods combines single-cell analyses of the epigenome and transcriptome. The simultaneous analysis of the epigenome and transcriptome enables scientists to investigate gene regulatory pathways. Accordingly, several approaches have been developed that profile chromatin accessibility and gene expression[45,46] as well as DNA methylation and gene expression.[41,47,48] In addition, commercially available solutions such as the "10x Chromium Single Cell Multiome ATAC + Gene expression" platform are now also available. Finally, a third type of multiomic methods enables the analysis of cellular proteins and the transcriptome profile of individual cells.[49,50] One example is the cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq). CITE-seq allows the detection of the protein of interest and the corresponding mRNA levels at single-cell resolution. It provides phenotypic information based on cell surface protein levels together with standard scRNAseq for an unbiased transcriptome analysis. It uses oligonucleotide-labeled antibodies to detect extracellular proteins and measures the transcriptome profile simultaneously, thus providing the joint information about abundance of protein and corresponding mRNA level.[49]

7 | SPATIAL TRANSCRIPTOMICS

Current single-cell sequencing methods require tissue dissociation and thus result in loss of spatial contextualisation. The emerging field of spatial transcriptomics addresses this problem by aiming to characterise gene expression profiles while retaining the spatial information of a tissue.[51] Spatial transcriptomics enables the visualisation of the mRNA distribution in tissue sections. Existing spatial transcriptomics methods can be roughly divided into (1) fluorescent in situ hybridization methods (e.g., RNAscope,[52] MERFISH,[53] seq-FISH[54] and (2) methods based on scRNA-seq (e.g., Slide-seq,[55] sci-Space[56]). Fluorescence-based methods rely on direct labelling of tissue sections with fluorescent probes[52–54] whereas scRNA-seq methods apply oligonucleotide or bead-based spatial barcoding.[55,56] Spatial transcriptomics at single-cell resolution is a rapidly developing field which provides a unique opportunity to dissect the tissue architecture together with underlying cellular interactions and thus offering a better understanding of morphologically complex tissues.[57] A commercially available Visium HD solution from 10x Genomics for single-cell spatial transcriptomics is planned to be released in the first half of 2022. Availability of an easy-to-use commercial product will increase the accessibility of spatial transcriptomics and further broaden its applications.

8 | COMPUTATIONAL METHODS FOR SINGLE-CELL ANALYSIS

The rapid growth and decreasing costs of single-cell methods is making them more accessible to scientists around the world.[28,58] The information about cellular phenotypes,
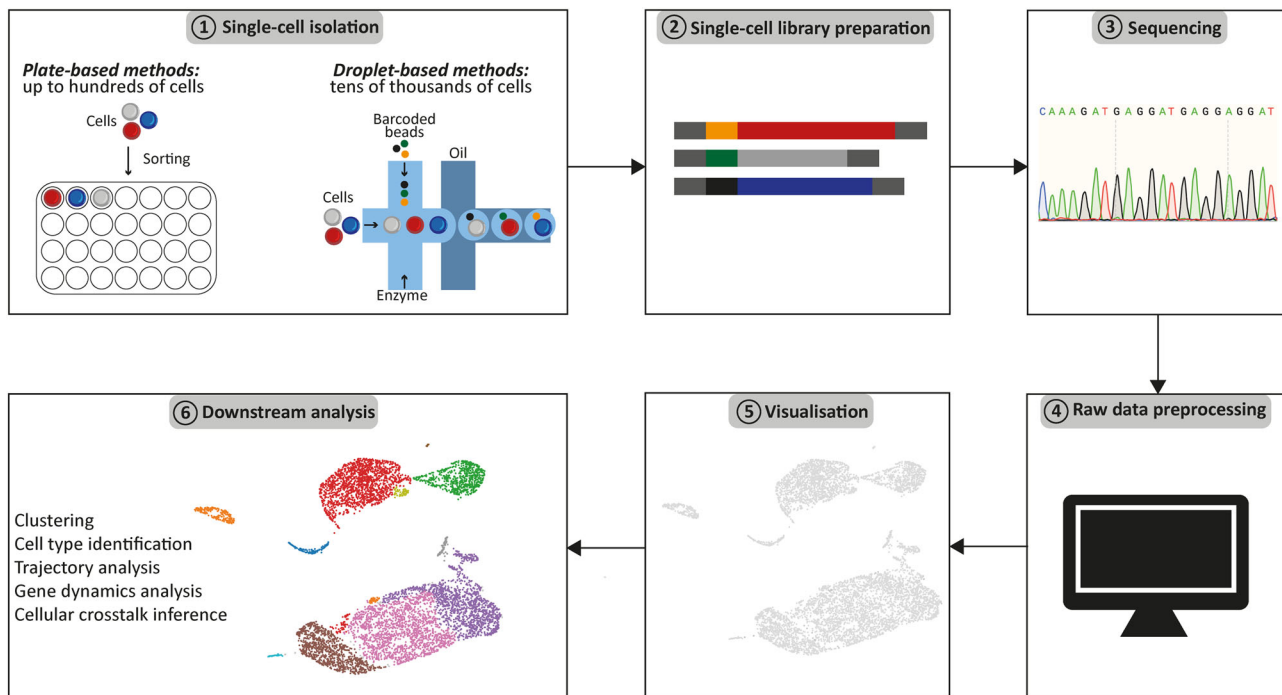
FIGURE 1 Workflow for scRNA-seq experiment. Single cells are isolated from heterogeneous tissue, followed by library preparation in which the barcoding step allows to distinguish material coming from each individual cell. Ready libraries are sequenced using Next Generation Sequencing methods. Raw sequencing data are processed using bioinformatic tools to generate count matrices followed by quality control. Dimensionality reduction methods are used for visualisation. Downstream analysis using appropriate bioinformatic tools allows to identify different cell types, perform trajectory analysis and/or infer cellular communication networks

developmental dynamics and communication networks is encrypted in a complex sequencing dataset. Current multiomics methods can measure multiple features of single cells, including the genome, transcriptome, epigenome and proteome. However, the implementation of computational methods is crucial to extract biological information from the data[59] and the volume and complexity of the data pose unique computational challenges. Single-cell data is characterised by a high level of technical noise and multifactorial variability between cells.[60,61] The widely recognised challenges within single-cell data science include (1) scaling up to higher dimensionalities regarding cells and features, (2) integration of single-cell data across different samples, experiments and modalities, and (3) validation and benchmarking of available analysis tools.[14]

Analysing single-cell data is especially challenging for first time users without strong bioinformatic support (Figure 1). Therefore, there is a growing focus on enhancing the user-friendliness and accessibility of available analytical tools.[62] Open source analysis tools with tutorials like Seurat from Satija's group (in R programming language)[63] or Scanpy from Theis's group (in Python programming language)[64] are available. In addition, Bioconductor (https://www.bioconductor.org/), an open source software for bioinformatics, provides sophisticated tools for the

analysis of high-throughput genomic data. Nevertheless, the application of those tools still requires basic programming skills. Therefore, commercial providers of single-cell technologies are developing softwares compatible with their products allowing easy interpretation of single-cell data for wet lab scientists without programming skills. One example of such software is Loupe Browser from 10x Genomics, which is a desktop application with point-and-click user interface that facilitates the visualisation and interpretation of data from different 10x Genomics solutions. This includes expression profile and/or chromatin accessibility of single-cells to subsequently identify and characterise cell types. Satija lab also developed a web application, Azimuth (https://satijalab.org/azimuth/) that uses publicly available data sets to facilitate the interpretation of scRNA-seq data. Azimuth uses the count matrix of gene expression from the single-cell experiment and performs data processing including normalisation and clustering. It is a reference-based mapping in which the uploaded dataset is compared to the reference dataset with annotated cell clusters to predict cluster identity. Further development of easy analysis tools will facilitate first interpretation of the data (Table 1). However, in-depth analysis of the complex dataset

TABLE 1 Examples of free single-cell data analysis tools

Name	Short description	Reference
Azimuth	Web application for quick interpretation of scRNA-seq data.	https://satijalab.org/azimuth/
Cell Ranger	Four pipelines to analyse data from different 10x Genomics solutions. Read alignements, generation of feature-barcode matrices, clustering, gene expression analysis.	10x Genomics
CogentAP	Processing of the sequencing data generated by ICELL8 technology including generation of gene matrix for downstream analysis.	Takara Bio
Cogenta NGS Discovery Software	Visualisation of the data generated by Cogenta AP.	Takara Bio
EpiScanpy	Preprocessing and analysis of epigenomic data (scATAC-seq and single-cell DNA methylation) including dimensionality reduction, clustering and visualisation.	Danese et al., (2019)[65]
Loupe Browser	Visualisation of the files generated by Cell Ranger pipelines.	10x Genomics
MultiMap	Tool for integration of scRNA-seq, scATAC-seq, single-cell DNA methylation and spatial data.	Jain et al., (2021)[66]
Scanpy	Preprocessing and analysis of scRNA-seq data including visualisation, clustering, trajectory interference, simulation of gene regulatory networks.	Wolf et al., (2018)[64]
Seurat	R package for quality control, analysis and exploration of scRNA-seq data.	https://satijalab.org/seurat/
Single Cell Interactive Application (SCiAp)	Tools and workflows for scRNA-seq data analysis from Human Cell Atlas and Single Cell Expression Atlas projects.	Moreno et al. (2021)[67] https://humancellatlas.usegalaxy.eu/
Singular Analysis Toolset Software	Analysis and visualisation of the data from Fluidigm systems. Gene expression profile and mutation patterns.	Fluidigm
Tapestri Insight	Software for single-cell DNA analysis and visualisation compatible with Tapestri Platform.	Mission Bio

requires good understanding of biological processes and advanced bioinformatics skills. It fosters the collaboration between dry and wet lab scientists.

9 | APPLICATION OF SINGLE-CELL TECHNOLOGIES

The rapid growth of single-cell methods is reflected in their increasing application in biology and medicine.[68] In general, the use of single-cell sequencing in this context can be roughly grouped into three main themes, (1) developmental studies,[69–71] (2) atlasing,[72] and (3) precision medicine.[73,74]

9.1 | Developmental studies

Single-cell approaches capture cells at various developmental stages, namely before, during and after lineage commitment, thus are well suited to resolve heterogeneity and steps during the developmental process. This approach was already successfully applied to study the development of nematodes,[70] mice[71] and zebrafish.[69] Single-cell methods are well suited to study the development of multicellular organisms as they can provide simultaneous measurement of clonal history and cell identity. In this context information about gene expression heterogeneity across differentiation pathways brings increased resolution to our understanding of the lineage commitment process.

9.2 | Atlasing

High-throughput single-cell methods are currently being applied to create a detailed map of the human organism under the initiative of the Human Cell Atlas (https://www.humancellatlas.org/). The scope is to create a detailed catalogue of all cell types present in the human body. The comparison of the cell types present during homeostasis with the diseased tissue will help to identify molecular changes

driving pathogenesis.[72] Additionally, the recent advances in mitochondrial DNA (mtDNA) tracing reveal the power of somatic mtDNA variation as natural genetic barcodes in primary human samples.[75] Using somatic mutation in mtDNA can be applied to trace clonal evolution of malignancies.

9.3 | Precision medicine

Highly innovative single-cell approaches are slowly starting their transition from basic science to clinical applications by enabling detailed characterization of cell types, states and pathways associated with human diseases. A single-cell multiomic approach with combined single-cell transcriptomics and lineage tracing was successfully applied to identify leukaemic and preleukaemic stem cells in acute myeloid leukaemia. It allowed the characterisation of the differentiation block stemming from the presence of leukaemic mutations.[74] Another great example illustrating the potential clinical application of single-cell methods, used a combination of whole exome sequencing and single-cell genotyping to understand genetic mechanisms driving progression and resistance in myelofibrosis.[73] There is an increased interest in potential clinical application of single-cell techniques reflected in collaborative initiatives like LifeTime. LifeTime aims to understand the complex behaviour of human cells during disease progression and analyse their response to the therapy, all at single-cell resolution.[76] To achieve that, further development and integration of multiomics methods is urgently needed. In the future, the drop in single-cell method costs and development of robust sample preprocessing pipelines will facilitate their transition to the clinic.

10 | CONCLUDING REMARKS

Single-cell methods are transforming our understanding of biological processes by allowing more complex profiling of cells forming an organism. Further technological developments will make it possible to obtain information on the past (genome mutations), present (transcriptome and proteome), and future (chromatin accessibility) state of a cell from a single experimental snapshot.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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REFERENCES

1. Chen G, Ning B, Shi T. Single-Cell RNA-Seq technologies and related computational data analysis. *Front Genet*. 2019;10:317. https://doi.org/10.3389/fgene.2019.00317
2. Method of the Year 2019: Single-cell multimodal omics. Method of the Year 2019: single-cell multimodal omics. *Nature Methods*. 2020;17(1):1-1. https://doi.org/10.1038/s41592-019-0703-5
3. Aldridge S, Teichmann SA. Single cell transcriptomics comes of age. *Nat Commun*. 2020;11(1):4307. https://doi.org/10.1038/s41467-020-18158-5
4. Lee J, Hyeon DY, Hwang D. Single-cell multiomics: technologies and data analysis methods. *Exp Mol Med*. 2020;52(9):1428-1442. https://doi.org/10.1038/s12276-020-0420-2
5. Paolillo C, Londin E, Fortina P. Single-cell genomics. *Clin Chem*. 2019;65(8):972-985. https://doi.org/10.1373/clinchem.2017.283895
6. Shema E, Bernstein BE, Buenrostro JD. Single-cell and single-molecule epigenomics to uncover genome regulation at unprecedented resolution. *Nature Genet*. 2018;51(1):19-25. https://doi.org/10.1038/s41588-018-0290-x
7. Luquette LJ, Bohrson CL, Sherman MA, Park PJ. Identification of somatic mutations in single cell DNA-seq using a spatial model of allelic imbalance. *Nat Commun*. 2019;10(1):1-14. https://doi.org/10.1038/s41467-019-11857-8
8. Mallory XF, Edrisi M, Navin N, Nakhleh L. Methods for copy number aberration detection from single-cell DNA-sequencing data. *Genome Biol*. 2020;21(1):208. https://doi.org/10.1186/s13059-020-02119-8
9. Woodworth MB, Girskis KM, Walsh CA. Building a lineage from single cells: genetic techniques for cell lineage tracking. *Nat Rev Genet*. 2017;18(4):230-244. https://doi.org/10.1038/nrg.2016.159
10. Kashima Y, Sakamoto Y, Kaneko K, Seki M, Suzuki Y, Suzuki A. Single-cell sequencing techniques from individual to multiomics analyses. *Exp Mol Med*. 2020;52(9):1419-1427. https://doi.org/10.1038/s12276-020-00499-2
11. Kester L, van Oudenaarden A. Single-cell transcriptomics meets lineage tracing. *Cell Stem Cell*. 2018;23(2):166-179. https://doi.org/10.1016/j.stem.2018.04.014
12. Liu J, Adhav R, Xu X. Current progresses of single cell DNA sequencing in breast cancer research. *Int J Biol Sci*. 2017;13(8):949-960. https://doi.org/10.7150/ijbs.19627
13. Zahn H, Steif A, Laks E, et al. Scalable whole-genome single-cell library preparation without preamplification. *Nature Methods*. 2017;14(2):167-173. https://doi.org/10.1038/nmeth.4140
14. Lähnemann D, Köster J, Szczurek E, et al. Eleven grand challenges in single-cell data science. *Genome Biol*. 2020;21(1):31. https://doi.org/10.1186/s13059-020-1926-6
15. Mincarelli L, Lister A, Lipscombe J, Macaulay IC. Defining cell identity with single-cell omics. *Proteomics*. 2018;18(18):e1700312. https://doi.org/10.1002/pmic.201700312

16. Tang F, Barbacioru C, Wang Y, et al. mRNA-Seq whole-transcriptome analysis of a single cell. *Nature Methods*. 2009; 6(5):377-382. https://doi.org/10.1038/nmeth.1315

17. Giladi A, Amit I. Single-cell genomics: a stepping stone for future immunology discoveries. *Cell*. 2018;172(1-2):14-21. https://doi.org/10.1016/j.cell.2017.11.011

18. Macosko EZ, Basu A, Satija R, et al. Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. *Cell*. 2015;161(5):1202-1214. https://doi.org/10.1016/j.cell.2015.05.002

19. Klein AM, Mazutis L, Akartuna I, et al. Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. *Cell*. 2015;161(5):1187-1201. https://doi.org/10.1016/j.cell.2015.04.044

20. Zheng GXY, Terry JM, Belgrader P, et al. Massively parallel digital transcriptional profiling of single cells. *Nat Commun*. 2017;8(1):14049. https://doi.org/10.1038/ncomms14049

21. Gierahn TM, Wadsworth MH, 2nd, Hughes TK, et al. Seq-Well: portable, low-cost RNA sequencing of single cells at high throughput. *Nature Methods*. 2017;14(4):395-398. https://doi.org/10.1038/nmeth.4179

22. Natarajan KN. Single-cell tagged reverse transcription (STRT-Seq). *Methods Mol Biol*. 2019;1979:133-153. https://doi.org/10.1007/978-1-4939-9240-9_9

23. Hagemann-Jensen M, Ziegenhain C, Chen P, et al. Single-cell RNA counting at allele and isoform resolution using Smart-seq. 3. *Nature Biotechnol*. 2020;38(6):708-714. https://doi.org/10.1038/s41587-020-0497-0

24. Picelli S, Faridani OR, Björklund AK, Winberg G, Sagasser S, Sandberg R. Full-length RNA-seq from single cells using Smart-seq. 2. *Nat Protoc*. 2014;9(1):171-181. https://doi.org/10.1038/nprot.2014.006

25. Ramsköld D, Luo S, Wang Y-C, et al. Full-length mRNA-Seq from single-cell levels of RNA and individual circulating tumor cells. *Nature Biotechnol*. 2012;30(8):777-782. https://doi.org/10.1038/nbt.2282

26. Hwang B, Lee JH, Bang D. Single-cell RNA sequencing technologies and bioinformatics pipelines. *Exp Mol Med*. 2018; 50(8):1-14. https://doi.org/10.1038/s12276-018-0071-8

27. Hayashi T, Ozaki H, Sasagawa Y, Umeda M, Danno H, Nikaido I. Single-cell full-length total RNA sequencing uncovers dynamics of recursive splicing and enhancer RNAs. *Nat Commun*. 2018;9(1): 1-16. https://doi.org/10.1038/s41467-018-02866-0

28. Svensson V, Vento-Tormo R, Teichmann SA. Exponential scaling of single-cell RNA-seq in the past decade. *Nat Protoc*. 2018;13(4):599-604. https://doi.org/10.1038/nprot.2017.149

29. van den Brink SC, Sage F, Vértesy Á, et al. Single-cell sequencing reveals dissociation-induced gene expression in tissue subpopulations. *Nature Methods*. 2017;14(10):935-936. https://doi.org/10.1038/nmeth.4437

30. Lambrechts D, Wauters E, Boeckx B, et al. Phenotype molding of stromal cells in the lung tumor microenvironment. *Nature Med*. 2018;24(8):1277-1289. https://doi.org/10.1038/s41591-018-0096-5

31. Cavalli G, Heard E. Advances in epigenetics link genetics to the environment and disease. *Nature*. 2019;571(7766):489-499. https://doi.org/10.1038/s41586-019-1411-0

32. Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. *Cell*. 2007;128(4):669-681. https://doi.org/10.1016/j.cell.2007.01.033

33. Buenrostro JD, Wu B, Litzenburger UM, et al. Single-cell chromatin accessibility reveals principles of regulatory variation. *Nature*. 2015;523(7561):486-490. https://doi.org/10.1038/nature14590

34. Satpathy AT, Granja JM, Yost KE, et al. Massively parallel single-cell chromatin landscapes of human immune cell development and intratumoral T cell exhaustion. *Nature Biotechnol*. 2019;37(8): 925-936. https://doi.org/10.1038/s41587-019-0206-z

35. Xu W, Wen Y, Liang Y, et al. A plate-based single-cell ATAC-seq workflow for fast and robust profiling of chromatin accessibility. *Nature Protocols*. 2021;16:4084-4107. https://doi.org/10.1038/s41596-021-00583-5

36. Grosselin K, Durand A, Marsolier J, et al. High-throughput single-cell ChIP-seq identifies heterogeneity of chromatin states in breast cancer. *Nature Genet*. 2019;51(6):1060-1066. https://doi.org/10.1038/s41588-019-0424-9

37. Bartosovic M, Kabbe M, Castelo-Branco G. Single-cell CUT&Tag profiles histone modifications and transcription factors in complex tissues. *Nature Biotechnol*. 2021;39(7): 825-835. https://doi.org/10.1038/s41587-021-00869-9

38. Ahn J, Heo S, Lee J, Bang D. Introduction to single-cell DNA methylation profiling methods. *Biomolecules*. 2021;11(7):1013. https://doi.org/10.3390/biom11071013

39. Smallwood SA, Lee HJ, Angermueller C, et al. Single-cell genome-wide bisulfite sequencing for assessing epigenetic heterogeneity. *Nature Methods*. 2014;11(8):817-820. https://doi.org/10.1038/nmeth.3035

40. Clark, S. J., Smallwood, S. A., Lee, H. J., Krueger, F., Reik, W., & Kelsey, G. (2017). Genome-wide base-resolution mapping of DNA methylation in single cells using single-cell bisulfite sequencing (scBS-seq). *Nature Protocols*, 12(3), 534–547. https://doi.org/10.1038/nprot.2016.187

41. Gu H, Raman AT, Wang X, et al. Smart-RRBS for single-cell methylome and transcriptome analysis. *Nature Protocols*. 2021;16:4004-4030. https://doi.org/10.1038/s41596-021-00571-9

42. Guo H, Zhu P, Guo F, et al. Profiling DNA methylome landscapes of mammalian cells with single-cell reduced-representation bisulfite sequencing. *Nat Protoc*. 2015;10(5): 645-659. https://doi.org/10.1038/nprot.2015.039

43. Gadalla R, Noamani B, MacLeod BL, et al. Validation of CyTOF against flow cytometry for immunological studies and monitoring of human cancer clinical trials. *Front Oncol*. 2019;9:415. https://doi.org/10.3389/fonc.2019.00415

44. Macaulay IC, Ponting CP, Voet T. Single-cell multiomics: multiple measurements from single cells. *Trends in Genetics: TIG*. 2017; 33(2):155-168. https://doi.org/10.1016/j.tig.2016.12.003

45. Cao J, Cusanovich DA, Ramani V, et al. Joint profiling of chromatin accessibility and gene expression in thousands of single cells. *Science*. 2018;361(6409):1380-1385. https://doi.org/10.1126/science.aau0730

46. Chen S, Lake BB, Zhang K. High-throughput sequencing of the transcriptome and chromatin accessibility in the same cell. *Nature Biotechnol*. 2019;37(12):1452-1457. https://doi.org/10.1038/s41587-019-0290-0

47. Clark SJ, Argelaguet R, Kapourani C-A, et al. scNMT-seq enables joint profiling of chromatin accessibility DNA methylation and transcription in single cells. *Nat Commun*. 2018; 9(1):1-9. https://doi.org/10.1038/s41467-018-03149-4

48. Niemöller C, Wehrle J, Riba J, et al. Bisulfite-free epigenomics and genomics of single cells through methylation-sensitive restriction. *Communications Biology.* 2021;4(1):1-10. https://doi.org/10.1038/s42003-021-01661-w

49. Stoeckius M, Hafemeister C, Stephenson W, et al. Simultaneous epitope and transcriptome measurement in single cells. *Nature Methods.* 2017;14(9):865-868. https://doi.org/10.1038/nmeth.4380

50. Triana SH, Vonficht D, Jopp-Saile L, et al. Single-cell proteogenomic reference maps of the hematopoietic system enable the purification and massive profiling of precisely defined cell states. *bioRxiv.* 2021. https://doi.org/10.1101/2021.03.18.435922

51. Burgess DJ. Spatial transcriptomics coming of age. *Nat Rev Genet.* 2019;20(6):317. https://doi.org/10.1038/s41576-019-0129-z

52. Wang F, Flanagan J, Su N, et al. RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. *J Mol Diagn.* 2012;14(1):22-29. https://doi.org/10.1016/j.jmoldx.2011.08.002

53. Chen KH, Boettiger AN, Moffitt JR, Wang S, Zhuang X. RNA imaging. Spatially resolved, highly multiplexed RNA profiling in single cells. *Science.* 2015;348(6233):aaa6090. https://doi.org/10.1126/science.aaa6090

54. Eng C-HL, Lawson M, Zhu Q, et al. Transcriptome-scale super-resolved imaging in tissues by RNA seqFISH. *Nature.* 2019;568(7751):235-239. https://doi.org/10.1038/s41586-019-1049-y

55. Rodriques SG, Stickels RR, Goeva A, et al. Slide-seq: a scalable technology for measuring genome-wide expression at high spatial resolution. *Science.* 2019;363(6434):1463-1467. https://doi.org/10.1126/science.aaw1219

56. Srivatsan SR, Regier MC, Barkan E, et al. Embryo-scale, single-cell spatial transcriptomics. *Science.* 2021;373(6550):111-117. https://doi.org/10.1126/science.abb9536

57. Garcia-Alonso L, Handfield L-F, Roberts K, et al. Mapping the temporal and spatial dynamics of the human endometrium in vivo and in vitro. *bioRxiv.* 2021. https://doi.org/10.1101/2021.01.02.425073

58. Stuart T, Satija R. Integrative single-cell analysis. *Nat Rev Genet.* 2019;20(5):257-272. https://doi.org/10.1038/s41576-019-0093-7

59. Haque A, Engel J, Teichmann SA, Lönnberg T. A practical guide to single-cell RNA-sequencing for biomedical research and clinical applications. *Genome Med.* 2017;9(1):75. https://doi.org/10.1186/s13073-017-0467-4

60. Efremova M, Teichmann SA. Computational methods for single-cell omics across modalities. *Nature Methods.* 2020;17(1):14-17. https://doi.org/10.1038/s41592-019-0692-4

61. Kharchenko PV, Silberstein L, Scadden DT. Bayesian approach to single-cell differential expression analysis. *Nature Methods.* 2014;11(7):740-742. https://doi.org/10.1038/nmeth.2967

62. Eisenstein M. Single-cell RNA-seq analysis software providers scramble to offer solutions. *Nature Biotechnol.* 2020;38(3):254-257. https://doi.org/10.1038/s41587-020-0449-8

63. Butler A, Hoffman P, Smibert P, Papalexi E, Satija R. Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nature Biotechnol.* 2018;36(5):411-420. https://doi.org/10.1038/nbt.4096

64. Wolf FA, Angerer P, Theis FJ. SCANPY: large-scale single-cell gene expression data analysis. *Genome Biol.* 2018;19(1):15. https://doi.org/10.1186/s13059-017-1382-0

65. Danese A, Richter ML, Fischer DS, Theis FJ. EpiScanpy: integrated single cell epigenomic analysis. *bioRxiv.* 2019. https://doi.org/10.1101/648097v1

66. Jain MS, Polanski K, Conde CD, et al. MultiMAP: dimensionality Reduction and Integration of Multimodal Data. *bioRxiv.* 2021. https://doi.org/10.1101/2021.02.16.431421

67. Moreno P., Huang N., Manning J. R., et al. User-friendly, scalable tools and workflows for single-cell RNAseq analysis. *Nature Methods.* 2021;18(4):327-328. https://doi.org/10.1038/s41592-021-01102-w

68. Strzelecka PM, Ranzoni AM, Cvejic A. Dissecting human disease with single-cell omics: application in model systems and in the clinic. *Dis Models & Mech.* 2018;11(11):dmm036525. https://doi.org/10.1242/dmm.036525

69. Alemany A, Florescu M, Baron CS, Peterson-Maduro J, van Oudenaarden A. Whole-organism clone tracing using single-cell sequencing. *Nature.* 2018;556(7699):108-112. https://doi.org/10.1038/nature25969

70. Packer JS, Zhu Q, Huynh C, et al. A lineage-resolved molecular atlas of C. elegans embryogenesis at single-cell resolution. *Science.* 2019;365(6459):eaax1971. https://doi.org/10.1126/science.aax1971

71. Pijuan-Sala B, Griffiths JA, Guibentif C, et al. A single-cell molecular map of mouse gastrulation and early organogenesis. *Nature.* 2019;566(7745):490-495. https://doi.org/10.1038/s41586-019-0933-9

72. Elmentaite R, Ross ADB, Roberts K, et al. Single-cell sequencing of developing human gut reveals transcriptional links to childhood crohn's disease. *Dev Cell.* 2020;55(6):771-783 e5. https://doi.org/10.1016/j.devcel.2020.11.010

73. Mylonas E, Yoshida K, Frick M, et al. Single-cell analysis based dissection of clonality in myelofibrosis. *Nat Commun.* 2020;11(1):73. https://doi.org/10.1038/s41467-019-13892-x

74. Velten L, Story BA, Hernández-Malmierca P, et al. Identification of leukemic and pre-leukemic stem cells by clonal tracking from single-cell transcriptomics. *Nat Commun.* 2021;12(1):1366. https://doi.org/10.1038/s41467-021-21650-1

75. Lareau CA, Ludwig LS, Muus C, et al. Massively parallel single-cell mitochondrial DNA genotyping and chromatin profiling. *Nature Biotechnol.* 2021;39(4):451-461. https://doi.org/10.1038/s41587-020-0645-6

76. Rajewsky N, Almouzni G, Gorski SA, et al. LifeTime Community Working Groups LifeTime and improving European healthcare through cell-based interceptive medicine. *Nature.* 2020;587(7834):377-386. https://doi.org/10.1038/s41586-020-2715-9