Lecture 8. Single-Cell and spatial omics technologies

1. Aim of the lecture. Expected learning outcomes

To introduce the principles of single-cell and spatial omics technologies, explain methods for analyzing molecular processes at cellular and tissue levels, and demonstrate their applications in studying cellular heterogeneity, gene regulation, development, environmental responses, and biotechnology.

2. Expected learning outcomes

1. Define single-cell omics and spatial omics.
2. Explain the limitations of conventional bulk omics.
3. Describe the main principles of single-cell RNA sequencing (scRNA-seq).
4. Distinguish between single-cell and single-nucleus sequencing.
5. Explain the principles of single-cell epigenomics and proteomics.
6. Describe the major approaches used in spatial transcriptomics.
7. Understand cell clustering, cell-type identification, and spatial mapping.
8. Explain the integration of single-cell and spatial omics datasets.
9. Discuss applications in medicine, biotechnology, and plant biology.

Lecture plan

Introduction: cellular heterogeneity and limitations of bulk omics

Single-cell transcriptomics: principles, technologies, workflow

Single-cell epigenomics, proteomics, and multi-omics

Spatial omics: concepts and major technologies

Bioinformatics and data integration

Applications in biotechnology and plant research

Introduction: cellular heterogeneity and limitations of bulk omics.
Challenges, conclusion, control questions, literature

Introduction to single-cell and spatial omics

Single-cell and spatial omics are advanced postgenomic technologies that enable the investigation of molecular processes at cellular resolution while revealing cellular heterogeneity and tissue organization.

1. Single-cell omics

- Analyzes gene expression, chromatin accessibility, proteins, or other molecular features in individual cells.
- Identifies distinct cell types, cellular states, and rare cell populations.
- Reveals molecular differences that are masked in conventional bulk tissue analysis.
- Includes single-cell transcriptomics (scRNA-seq), epigenomics, and proteomics.

Example in plant biotechnology: Single-cell and spatial transcriptomics can reveal cell-type-specific gene expression in wheat roots, helping investigate nutrient transport, root development, and responses to drought or salinity.

2. Spatial omics

- Investigates the distribution of molecular features within their original tissue context.
- Preserves information about cellular localization and tissue architecture.
- Enables analysis of spatial gene expression patterns and relationships between neighboring cells.
- Includes spatial transcriptomics, spatial proteomics, and spatial metabolomics.
- Major advantages:
- High-resolution characterization of cellular diversity.
- Identification of cell-type-specific molecular pathways.
- Investigation of developmental processes and responses to environmental stress.
- Integration of molecular information with tissue structure.

- *Key concept: Single-cell omics reveals which cells express particular molecular features, while spatial omics reveals where these molecular activities occur within tissues.*

Why is single-cell analysis important?

Traditional bulk analysis measures the average molecular signal from many cells.

However, biological tissues contain different cell types and cellular states.

For example, a plant leaf includes:

Mesophyll cells.

Epidermal cells.

Guard cells.

Vascular cells.

Other specialized cell populations.

These cells differ in gene expression, metabolism, and physiological function.

Single-cell analysis reveals this heterogeneity.

Bulk omics versus single-cell omics

Feature	Bulk omics	Single-cell omics
Biological material	Mixed cell population	Individual cells
Gene expression	Average signal	Cell-specific profiles
Rare cell populations	May be masked	Can be detected
Cell-type identification	Limited	High resolution
Data complexity	Moderate to high	Very high
Spatial information	Usually lost	Usually lost unless spatially integrated

4. Cellular heterogeneity

Cellular heterogeneity refers to differences in molecular composition, gene expression, functional activity, or physiological state among cells.

Two important forms are:

1. Cell-type heterogeneity: differences between specialized cell types.
2. Cell-state heterogeneity: differences between cells of the same type under different developmental or environmental conditions.

These differences are fundamental to development, tissue organization, and stress responses.

From bulk omics to spatially resolved biology

The evolution from bulk omics to single-cell and spatial omics has transformed molecular biology by enabling the investigation of cellular heterogeneity and the spatial organization of biological processes.

1. Bulk omics: Tissue-level analysis

- Measures average molecular signals from large populations of cells.
- Provides information about overall gene expression, protein abundance, and metabolic profiles.
- Cannot directly distinguish molecular differences between individual cell types.
- May mask rare cell populations and cell-specific responses.

2. Single-cell omics: Cellular-level analysis

- Characterizes molecular profiles of individual cells or nuclei.
- Identifies distinct cell types, cellular states, and rare populations.
- Reveals cellular heterogeneity and developmental transitions.
- Usually loses the original spatial positions of cells during sample dissociation.

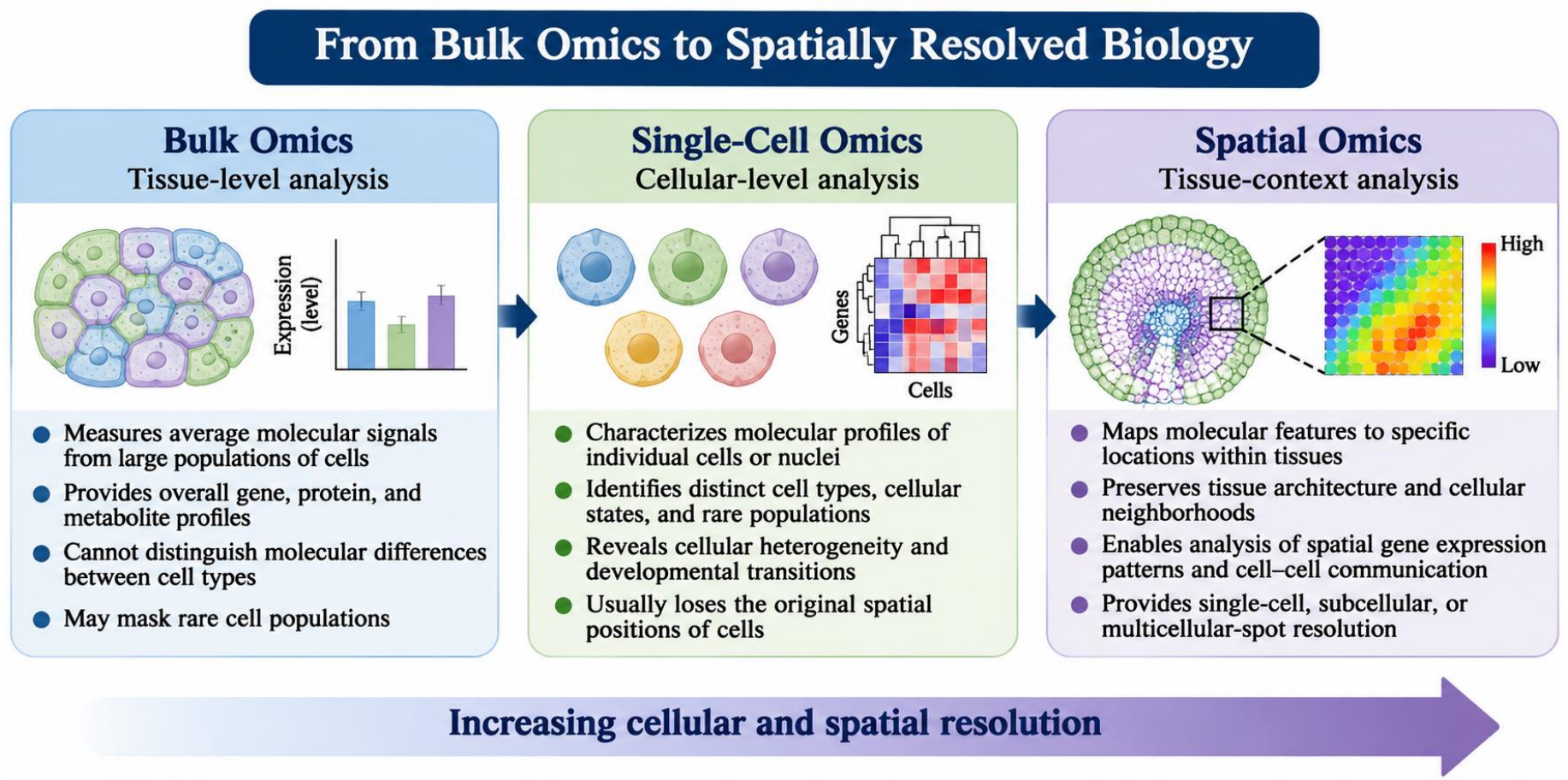
3. Spatial omics: Tissue-context analysis

- Maps molecular features to specific locations within intact or sectioned tissues.
- Preserves information about tissue architecture and cellular neighborhoods.
- Enables investigation of spatial gene expression patterns and potential cell–cell communication.
- Depending on the technology, provides single-cell, subcellular, or multicellular-spot resolution.

Example in plant biotechnology: In wheat roots, bulk RNA-seq measures average gene expression, single-cell RNA-seq identifies cell-type-specific transcriptional responses, and spatial transcriptomics reveals their distribution across root tissues.

Key concept: The integration of bulk, single-cell, and spatial omics provides complementary information, connecting tissue-level molecular changes with cellular identity and spatial organization.

From bulk omics to spatially resolved biology



Single-cell transcriptomics

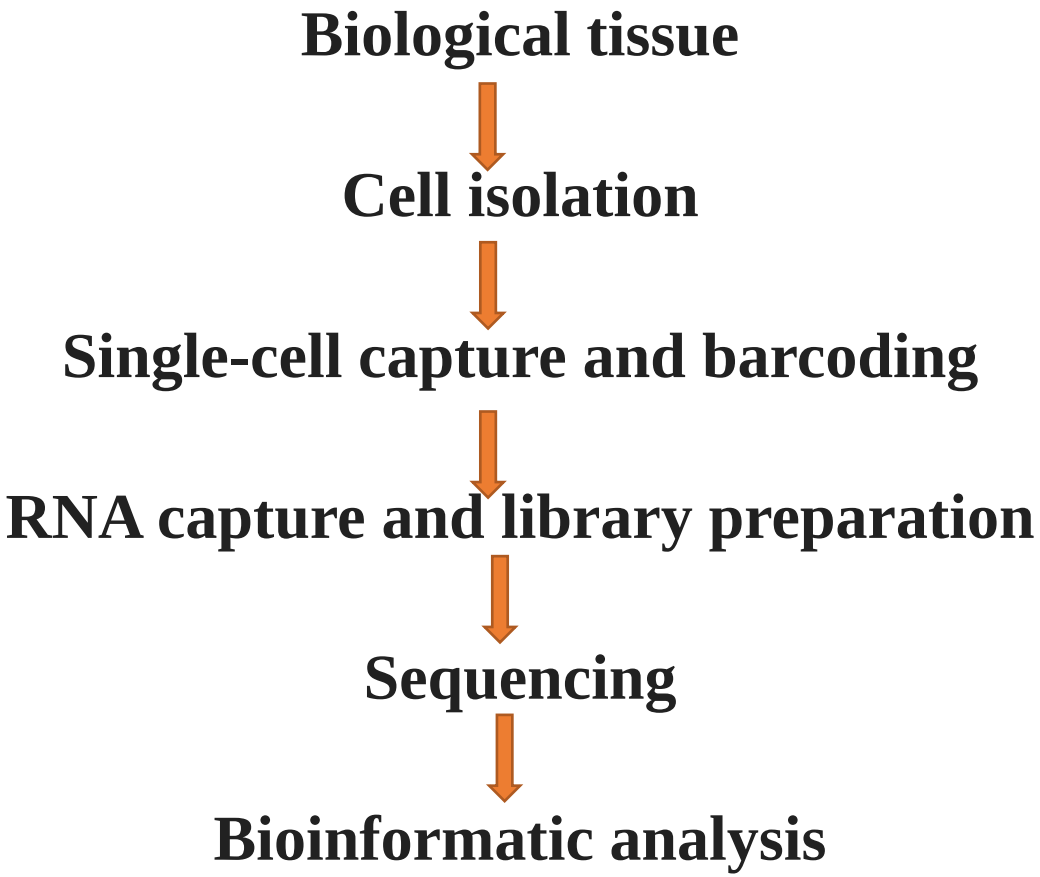
Single-cell RNA sequencing (scRNA-seq)

scRNA-seq is a technology used to profile RNA expression in individual cells.

It allows researchers to:

1. **Identify different cell populations.**
2. **Characterize cell-specific gene-expression patterns.**
3. **Detect rare cell types.**
4. **Investigate developmental trajectories.**
5. **Study responses to stress and disease.**

General scRNA-seq workflow



Technologies for single-cell isolation

Major approaches include:

1. **Fluorescence-activated cell sorting (FACS).**
2. **Microfluidic droplet-based systems.**
3. **Microwell-based capture.**
4. **Manual micromanipulation.**
5. **Laser capture microdissection for selected tissue regions or cells.**

The choice depends on cell size, viability, tissue properties, and experimental objectives.

Droplet-based single-cell sequencing

Droplet-based single-cell sequencing is a high-throughput technology that uses microfluidic systems to isolate individual cells or nuclei in microscopic droplets for molecular profiling.

Principle of the technology:

- Microfluidic encapsulation: Individual cells or nuclei are captured in droplets together with barcoded beads.
- Cell-specific barcoding: Each bead carries oligonucleotides containing a cell barcode and unique molecular identifiers (UMIs).
- Cell lysis and RNA capture: RNA molecules are released and captured by bead-associated primers.
- Library preparation: Barcoded cDNA is generated, amplified, and prepared for sequencing.
- Bioinformatics analysis: Sequencing reads are assigned to individual cells to construct cell-by-gene expression matrices.

Droplet-based single-cell RNA-seq workflow

Cell/nuclei isolation **Droplet formation** **RNA capture**

Sample preparation **Cell + barcoded bead** **Cell barcodes + UMIs**

Sequencing **- Barcoded libraries**

Data analysis **Cell expression profiles**

Major advantages:

- Simultaneous profiling of thousands of cells or nuclei.
- Identification of rare cell populations and distinct cellular states.
- High-throughput characterization of cellular heterogeneity.
- Compatibility with computational cell clustering and developmental trajectory analysis.

Limitations:

- Cell dissociation may introduce stress-related transcriptional changes.
- Doublets and ambient RNA contamination can affect data quality.
- Low-abundance transcripts may not be detected consistently.
- Original spatial information is generally lost.

Key concept: Droplet-based sequencing enables high-throughput molecular profiling of individual cells or nuclei, revealing cellular diversity that is masked in bulk transcriptomic analysis.

Important distinction: Droplet-based platforms are most commonly used for single-cell or single-nucleus RNA sequencing; related microfluidic approaches can also support other single-cell molecular assays

10. Cell barcodes and unique molecular identifiers

Two important concepts:

1. Cell barcode: identifies the cell from which a sequencing read originated.
2. Unique molecular identifier (UMI): labels individual captured RNA molecules to help distinguish original molecules from PCR duplicates.

These approaches improve the reliability of gene-expression quantification.

11. Main results of scRNA-seq

The major output is a gene-expression matrix:

Genes × Cells

This matrix is used for:

1. **Cell clustering.**
2. **Cell-type annotation.**
3. **Identification of marker genes.**
4. **Differential expression analysis.**
5. **Developmental trajectory inference.**

Single-cell epigenomics and proteomics

Single-cell epigenomics and proteomics extend molecular analysis beyond gene expression by investigating chromatin regulation, protein abundance, and cellular functions at individual-cell resolution.

1. Single-cell epigenomics

- scATAC-seq: Identifies accessible chromatin regions and potential regulatory elements in individual cells.
- Single-cell DNA methylation sequencing: Characterizes cell-specific DNA methylation patterns.
- scCUT&Tag: Profiles selected histone modifications or chromatin-associated proteins at single-cell resolution.
- Multiome approaches: Simultaneously analyze gene expression and chromatin accessibility in the same cell or nucleus.

2. Single-cell proteomics

- Mass cytometry (CyTOF): Quantifies multiple proteins using metal-labeled antibodies and mass spectrometric detection.
- Single-cell mass spectrometry: Enables increasingly sensitive identification and quantification of proteins from individual cells.
- Antibody-based protein profiling: Measures selected cell-surface or intracellular proteins.
- CITE-seq: Combines single-cell RNA sequencing with antibody-derived measurements of selected proteins.

Major applications:

- Identification of cell-specific regulatory mechanisms.
- Characterization of cellular differentiation and developmental processes.
- Investigation of cellular responses to environmental stress.
- Integration of epigenomic, transcriptomic, and proteomic information.

Single-cell epigenomics and proteomics

Comparison of single-cell technologies

echnology	Molecular information	Main application
scATAC-seq	Chromatin accessibility	Regulatory element identification
scCUT&Tag	Histone marks/protein occupancy	Epigenetic regulation
CyTOF	Selected protein abundance	Cellular phenotyping
Single-cell MS	Protein abundance	Proteome characterization
ITE-seq	RNA + selected proteins	Multimodal cell profiling

Example in plant biotechnology: Single-nucleus ATAC-seq combined with transcriptomics can help identify regulatory regions associated with cell-type-specific gene expression during wheat root development and responses to drought stress.

Key concept: *Integrating single-cell epigenomics and proteomics provides complementary insights into how chromatin regulation and protein expression contribute to cellular identity and function.*

Methodological consideration: Antibody-based approaches measure selected protein targets, whereas mass spectrometry-based methods can provide broader protein coverage but remain technically challenging at single-cell scale.

13. Single-cell ATAC-seq

scATAC-seq (Assay for Transposase-Accessible Chromatin with sequencing) identifies accessible chromatin regions in individual cells or nuclei.

It can help reveal:

1. **Putative regulatory elements.**
2. **Cell-type-specific chromatin accessibility.**
3. **Transcription-factor binding motifs.**
4. **Regulatory differences between cellular populations.**

Single-cell proteomics. Single-cell multi-omics

Single-cell proteomics aims to measure proteins in individual cells.

Major approaches include:

1. **Antibody-based protein detection.**
2. **Mass cytometry.**
3. **Single-cell mass-spectrometry proteomics.**
4. **Combined RNA and surface-protein profiling.**

Compared with scRNA-seq, comprehensive single-cell proteomics remains technically challenging because proteins cannot be amplified like nucleic acids.

Single-cell multi-omics combines multiple molecular measurements at cellular resolution.

Examples:

1. RNA + chromatin accessibility
2. RNA + surface proteins
3. DNA methylation + gene expression

Integration helps connect regulatory states with molecular function.

Spatial omics technologies. Spatial transcriptomics

What are spatial omics?

Spatial omics are technologies that analyze molecular characteristics while preserving or reconstructing their location within biological tissues.

Spatial omics can reveal:

1. ***Tissue organization.***
2. ***Spatial gene-expression patterns.***
3. ***Molecular differences between tissue regions.***
4. ***Cell neighborhoods.***
5. ***Potential cell–cell communication.***

Spatial transcriptomics measures RNA expression together with information about its location in tissue.

Unlike conventional scRNA-seq, it retains spatial context.

Two broad technological strategies are:

1. **Sequencing-based spatial transcriptomics.**
2. **Imaging-based spatial transcriptomics.**

Sequencing-based spatial transcriptomics

- In capture-based methods, tissue sections are placed onto surfaces containing spatially barcoded probes.
- RNA molecules are captured and assigned to defined spatial positions.
- Single-cell and spatial transcriptomics: deciphering brain complexity in health and disease | Nature Reviews Neurology
- Depending on platform resolution, one spatial measurement may represent one cell, part of a cell, or multiple cells.

19. Imaging-based spatial transcriptomics

Imaging-based methods detect RNA molecules directly within fixed cells or tissue sections.

Examples include:

1. *Multiplexed fluorescence in situ hybridization (MERFISH).*
2. *Sequential fluorescence in situ hybridization (seqFISH).*
3. *Other highly multiplexed RNA imaging approaches.*

- These methods can provide high spatial resolution and detailed localization of selected RNA targets.

Methods in spatial proteomics

Spatial proteomics investigates protein distributions and interactions within tissue architecture.

Methods include:

1. **Multiplex immunofluorescence.**
2. **Imaging mass cytometry.**
3. **Mass-spectrometry imaging.**
4. **Spatially resolved antibody-based profiling.**

These approaches help characterize tissue organization and protein localization.

Single-cell versus spatial omics

Feature	Single-cell omics	Spatial omics
Primary focus	Individual cell molecular profiles	Molecular profiles and tissue location
Cell isolation	Often required	Often not required
Spatial context	Usually lost	Preserved or reconstructed
Main strength	Cellular heterogeneity	Tissue organization
Major application	Cell types and states	Spatial regulation and tissue interactions

5. Bioinformatics and data integration

Single-cell data-processing workflow

Raw sequencing data

↓

Quality control

↓

Read alignment or mapping

↓

Gene-count matrix

↓

Normalization

↓

Dimensionality reduction

↓

Cell clustering

↓

Cell-type annotation

↓

Biological interpretation

Dimensionality reduction: PCA, UMAP and t-SNE

Single-cell datasets contain thousands of gene-expression measurements per cell.

Dimensionality reduction enables visualization of complex cellular populations.

Common methods:

1. **Principal component analysis (PCA).**
2. **Uniform Manifold Approximation and Projection (UMAP).**
3. **t-distributed Stochastic Neighbor Embedding (t-SNE).**

- Research suggests how tumors become aggressive prostate cancer | Cornell Chronicle

Important: Distances between groups on UMAP or t-SNE plots should not automatically be interpreted as exact biological distances.

Cell clustering and annotation

Cells are grouped according to similarities in their molecular profiles.

Clusters are annotated using:

1. **Known marker genes.**
2. **Reference cell atlases.**
3. **Differential gene expression.**
4. **Functional enrichment.**
5. **Biological knowledge of the tissue.**

Cell annotation should be supported by multiple lines of evidence.

Challenges and Future Perspectives in Single-Cell and Spatial Omics

Single-cell and spatial omics technologies are transforming postgenomic research by enabling high-resolution investigation of cellular heterogeneity, tissue organization, and molecular regulation.

Major challenges:

- Sample preparation: Difficulties in isolating viable cells or intact nuclei, particularly from plant tissues with rigid cell walls.
- Technical variability: Low RNA capture efficiency, dropout events, doublets, and batch effects.
- Spatial resolution: Trade-offs between molecular coverage, sensitivity, and spatial precision.
- Data complexity: Large datasets requiring advanced bioinformatics and computational resources.
- Biological interpretation: Challenges in identifying cell types, reconstructing developmental trajectories, and validating cell–cell interactions.

Future research directions:

- Single-cell multi-omics: Simultaneous profiling of transcriptomic, epigenomic, and selected proteomic features.
- High-resolution spatial transcriptomics: Improved mapping of gene expression at cellular and subcellular resolution.
- Spatial proteomics and metabolomics: Investigation of protein and metabolite distributions within tissues.
- AI-assisted analysis: Automated cell annotation, spatial pattern recognition, and multimodal data integration.
- Plant biotechnology: Development of cell-type-specific molecular maps to support crop improvement and stress research.

Key concept: Integration of single-cell, spatial, and multi-omics technologies will enable increasingly comprehensive analysis of biological processes at cellular and tissue levels.

Control Questions

1. What are single-cell and spatial omics, and how do they differ from bulk omics?
2. What are the major advantages of single-cell RNA sequencing?
3. How does droplet-based single-cell sequencing work?
4. What are cell barcodes and unique molecular identifiers (UMIs)?
5. What are the main applications of single-cell epigenomics and proteomics?
6. How do scATAC-seq and single-cell RNA-seq provide complementary information?
7. What are the major principles and technologies of spatial transcriptomics?
8. How are cell clustering, dimensionality reduction, and trajectory analysis applied to single-cell data?
9. What challenges are associated with single-cell analysis of plant tissues?
10. How can single-cell and spatial omics contribute to plant biotechnology and crop improvement?

Conclusion

- Single-cell omics reveals molecular heterogeneity among individual cells and nuclei.
- Spatial omics maps molecular features within their original tissue context.
- Droplet-based sequencing enables high-throughput characterization of cellular transcriptomes.
- Single-cell epigenomics and proteomics provide complementary information about chromatin regulation and protein expression.
- Spatial transcriptomics, proteomics, and metabolomics reveal tissue-specific molecular organization.
- Bioinformatics methods support cell clustering, cell-type identification, developmental trajectory inference, and spatial data analysis.
- Integration of single-cell and spatial omics advances understanding of development, environmental responses, and cellular interactions.

Overall conclusion: Single-cell and spatial omics connect molecular regulation with cellular identity and tissue organization, providing powerful approaches for functional genomics, systems biology, and modern biotechnology.

Recommended Literature

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