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scDiagnostics: diagnostic functions to assess the quality of cell type annotations in single-cell RNA-Seq data

scDiagnostics Package Authors

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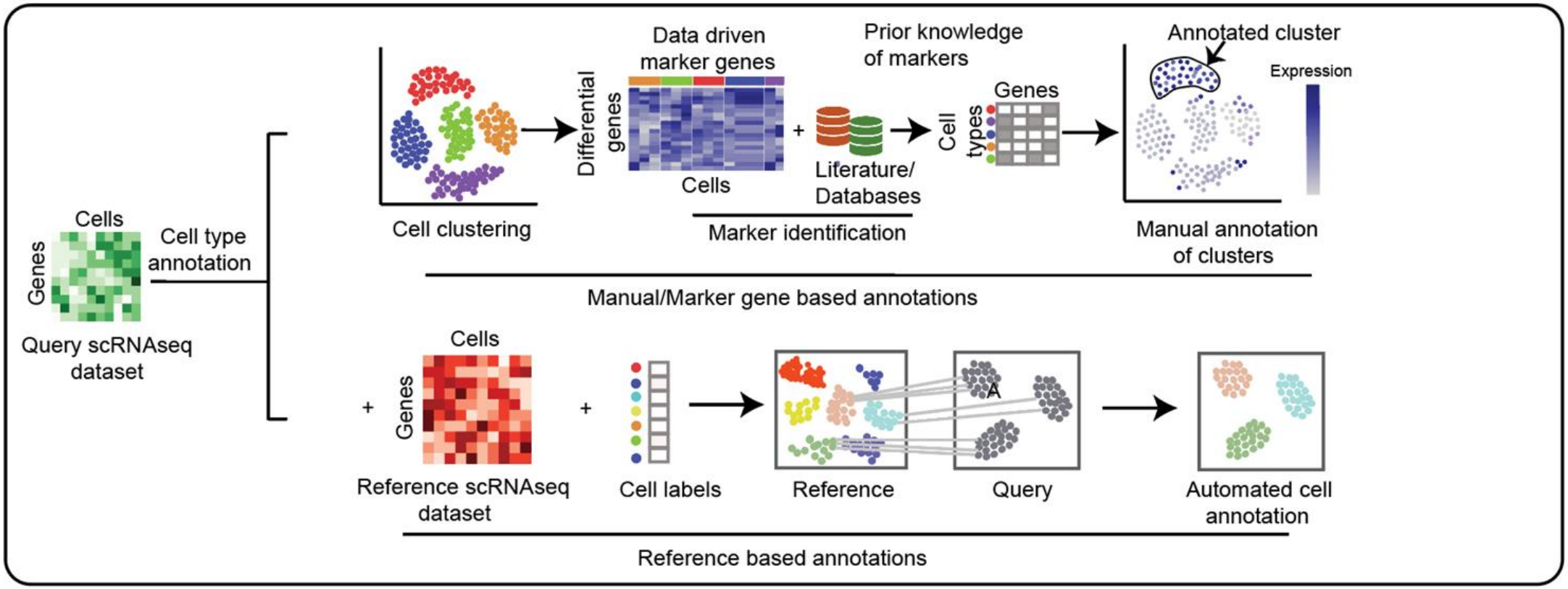
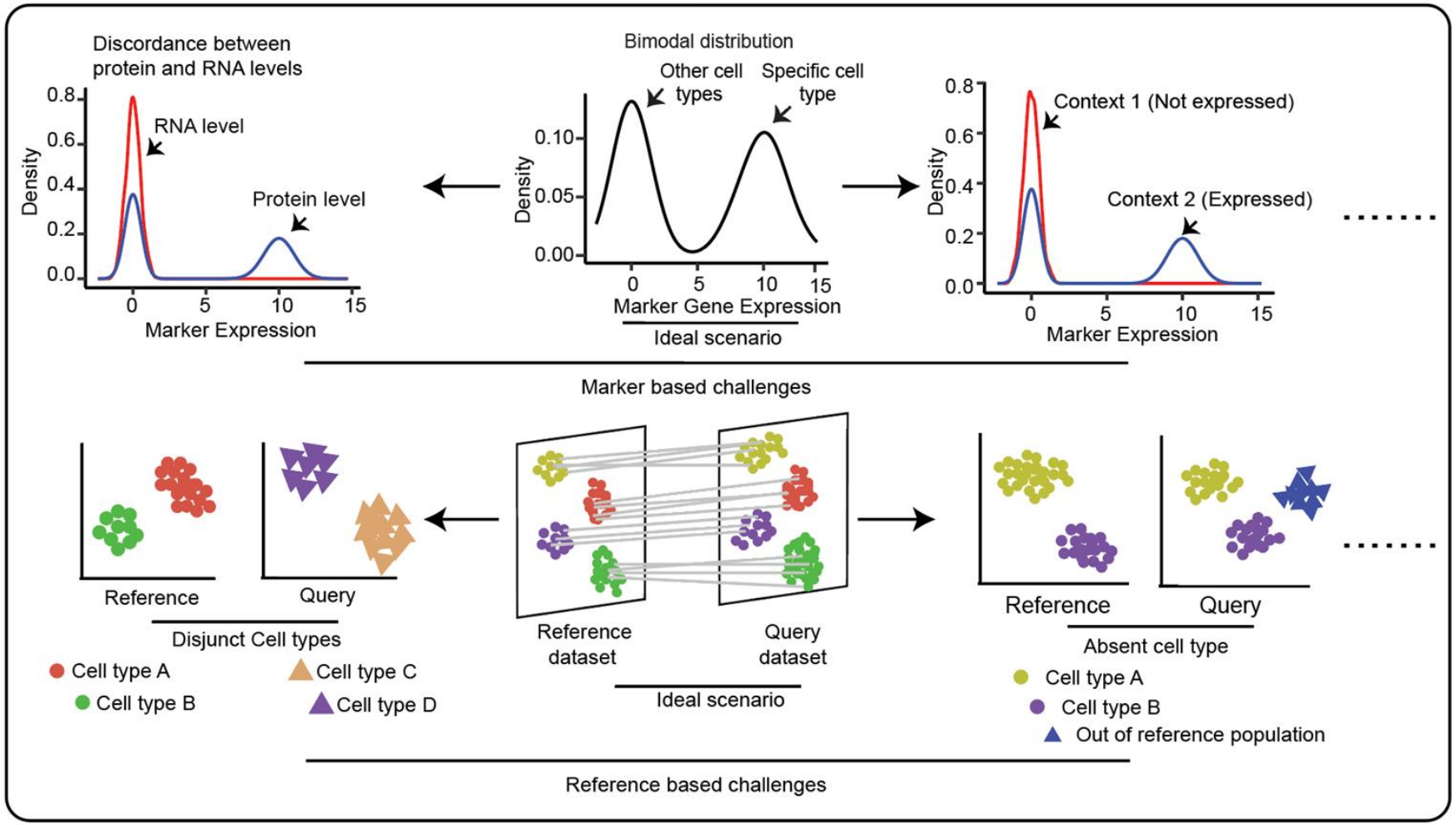
Background

Two Main Approaches to Cell Type Annotation



Background

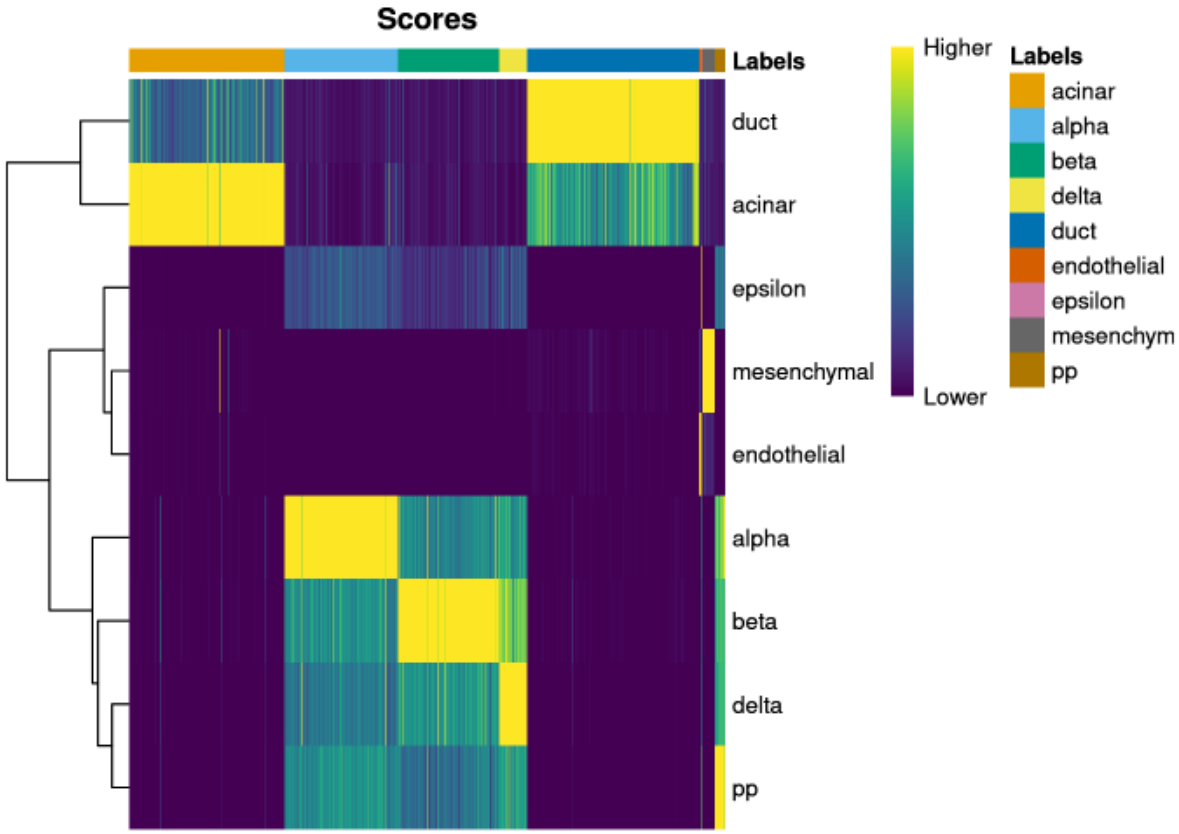
Undiagnosed Challenges in Cell Type Annotation



Previous Work

Annotation Diagnostics in SingleR

- **SingleR Annotation Diagnostics:**
  - Chapter 4 of SingleR book
- **Limited Scope:**
  - If some cell types are unique to query dataset, an annotation score is still given.
  - If a cell has an ambiguous cell type assignment, it is hard to see why.



Data

Reference and Query Datasets

- **Reference Data:**
  - Well-curated & expert-annotated
  - Cells have been labeled by domain experts using known marker genes and experimental validation (sometimes…)
  - Serves as ground truth for cell type annotation transfer
  - Used to train models, identify cell types, and serve as benchmark data for new annotation methods.
  - **Reference datasets in the `celldex` package:**
    - **Human Cell Atlas (HCA):** >100,000 cells from 15 human tissues
    - **Mouse Cell Atlas (MCA):** >100,000 cells from 14 mouse tissues.
    - **Human Peripheral Blood Mononuclear Cells (PBMCs):** >20,000 PBMCs from healthy donors, covering various immune cell types
    - And many more…

Data

Reference and Query Datasets

- **Query Data:**
  - new single-cell RNA-seq dataset that needs to be analyzed / annotated
  - cell types have been annotated by some method (e.g. annotation transfer), but their accuracy as not been assessed
  - **Goals:**
    - **Dataset alignment:** Ensure that query data aligns well with the reference data.
    - **Annotation Validation:** Assess to which extent the query cells have been well annotated.
    - **Anomalous Cell Detection:** Identify any potentially anomalous cells that may indicate issues with data quality or annotation.

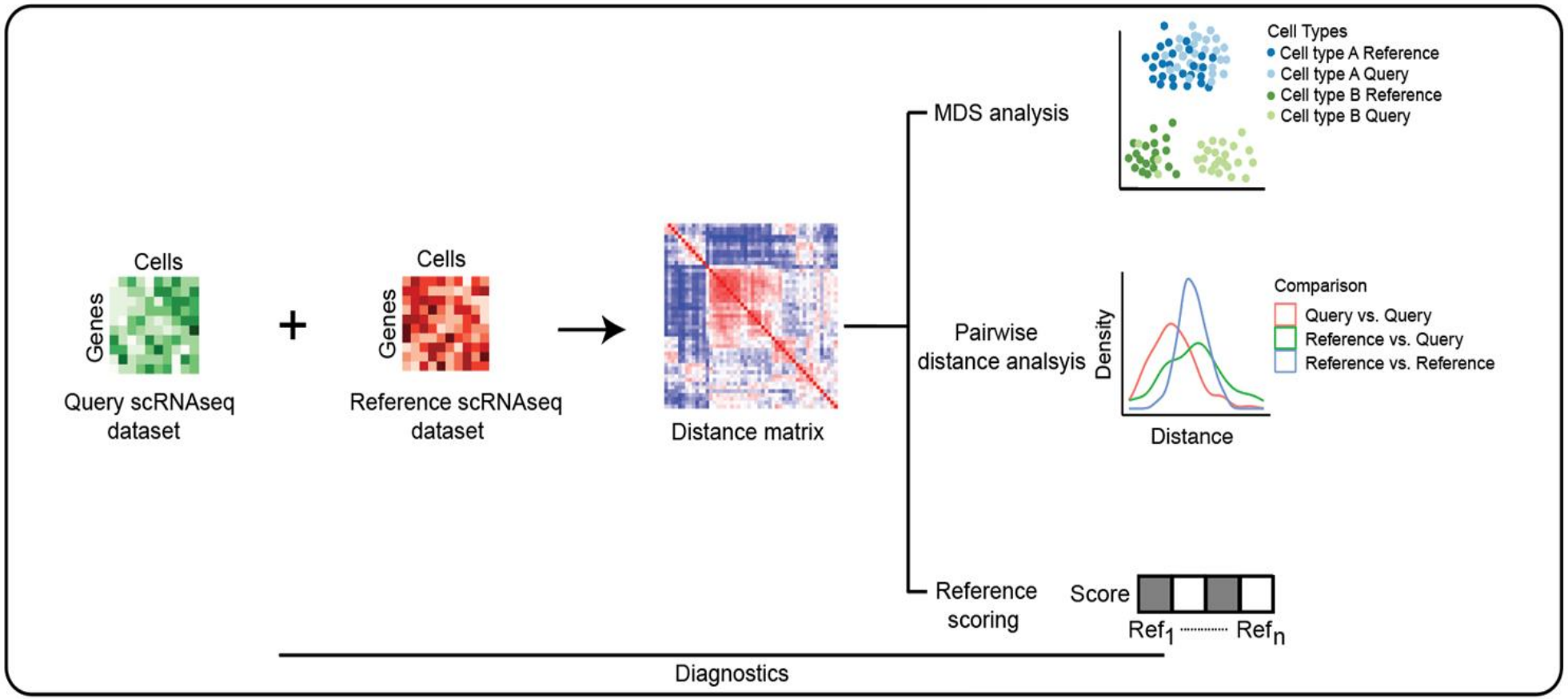
Introducing scDiagnostics

Enhancing the Standard scRNA-seq Cell Annotation Pipeline

- **Core Functionality:**
  - **Data Alignment Checking:** Ensures that the query dataset is well aligned with the reference dataset, verifying the consistency of gene expression patterns.
    - E.g. PCA subspace comparison, canonical correlation analysis, Wasserstein Distances.
  - **Annotation Validation:** Confirms the accuracy of cell type annotations in the query dataset, using statistical and computational methods.
    - E.g. discriminant space projections, PCA projections, distance and correlation-based analyses.
  - **Anomalous Cell Detection:** Identifies potentially anomalous cells, ensuring robust and accurate results.
    - E.g. isolation forests, direction of outlyingness (future work).
- **Impact:**
  - Enhances the reliability and accuracy of scRNA-seq data analysis.
  - Provides confidence in the results obtained from downstream analysis.

Introducing scDiagnostics

Enhancing the Standard scRNA-seq Cell Annotation Pipeline



Example

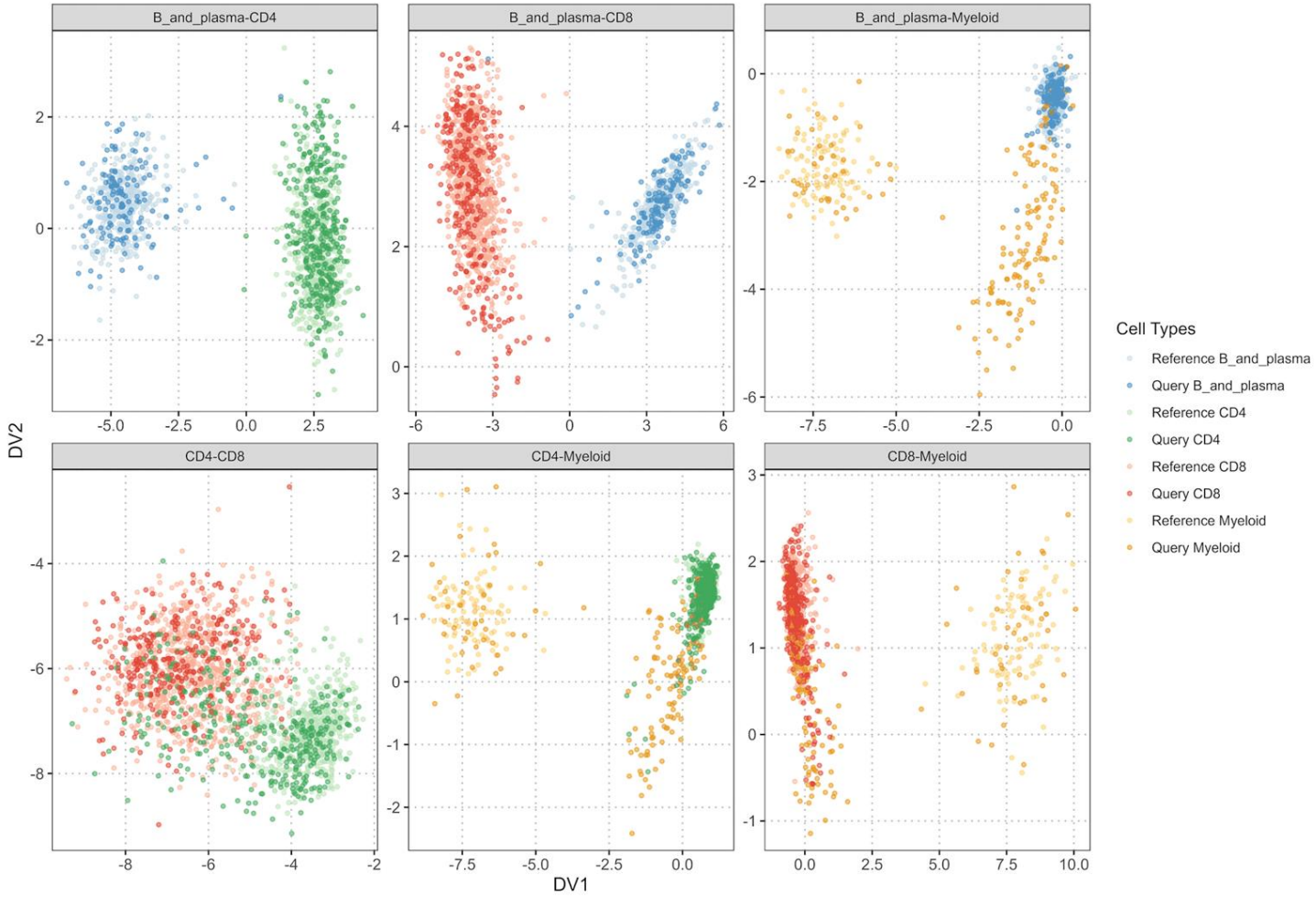
Cell Type Annotation With SingleR

- `HeOrganAtlasData(tissue = c("Marrow"))` from the `scRNAseq` package
- Standard OSCA-based preprocessing (QC, normalization, etc.)
- 70% of data as “reference” and remaining 30% as “query” data
- Annotation transfer from reference to query via `SingleR`

	SingleR Annotation			
Expert Annotation	B and Plasma	CD4	CD8	Myeloid
B and Plasma	139	0	0	10
CD4	0	190	1	13
CD8	0	134	196	29
Myeloid	0	0	0	40

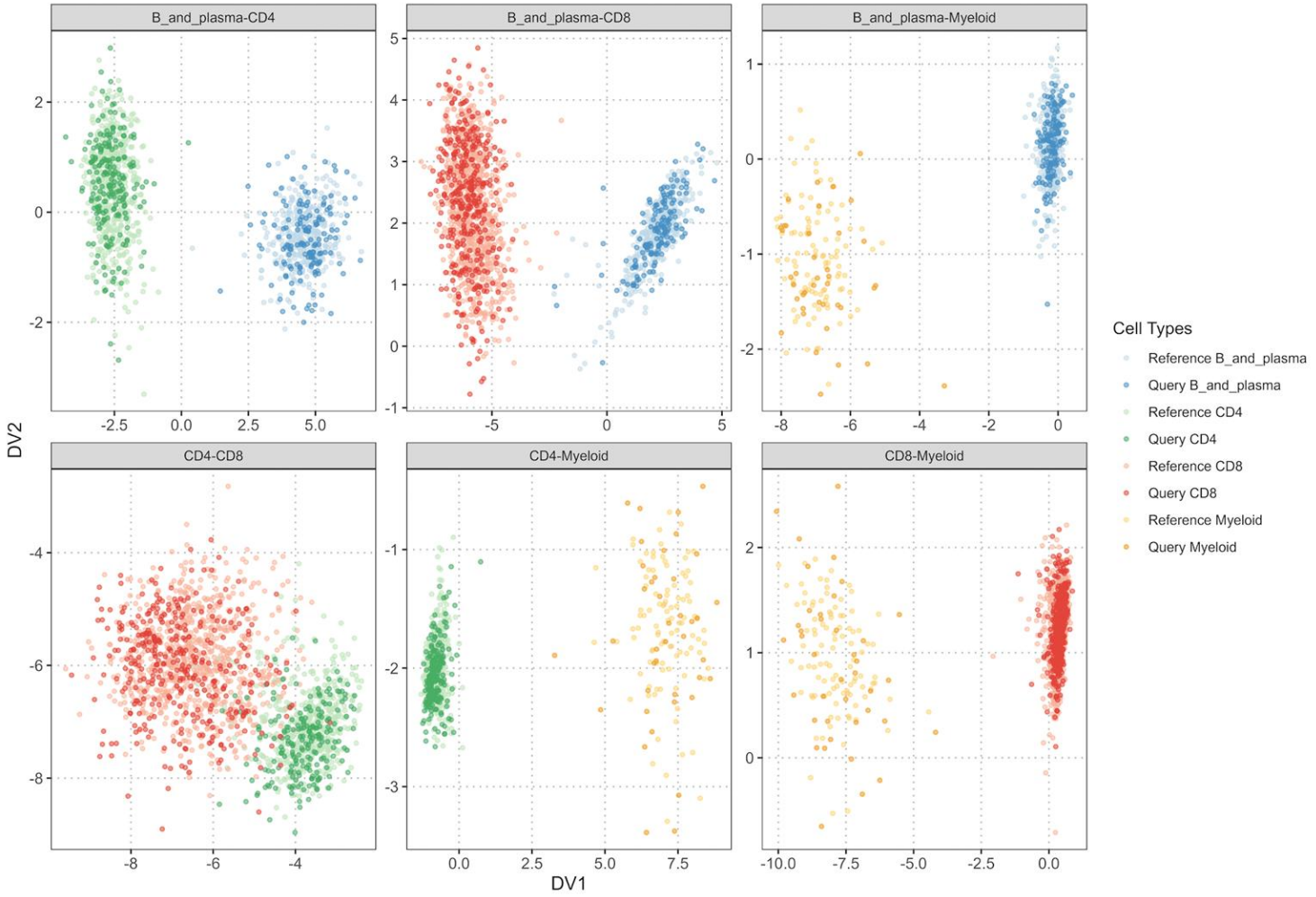
Visualization of SingleR Annotation

“Poor” Annotation in Discriminant Space



Visualization of Expert Annotation

“Good” Annotation in Discriminant Space



Workshop Materials

Package Information and Links

- **Package (development) GitHub repository**
- **Package website**
- **Workshop Materials:**
  - Repository
  - Slides
  - Vignette
  - Docker image
  - Galaxy workshop
- **Package will soon be available on Bioconductor.**

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