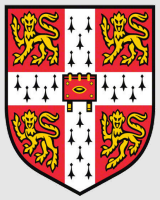

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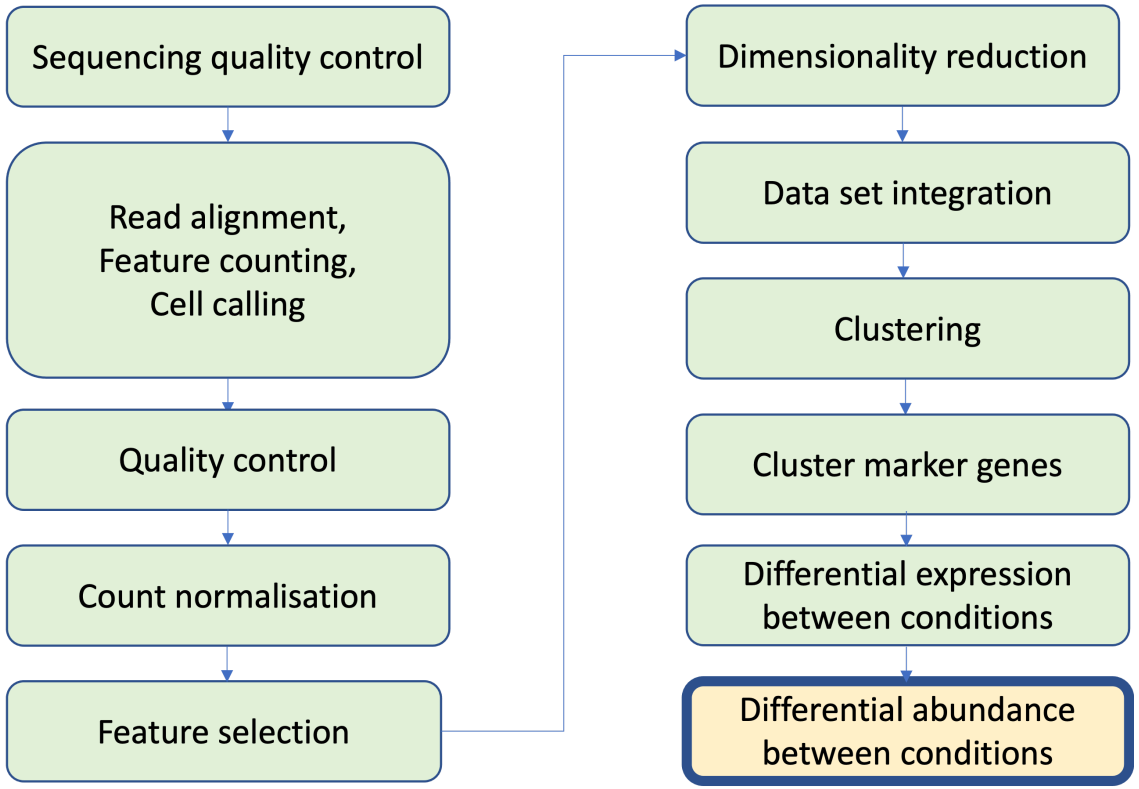
Differential Abundance Analysis

February 2026

Single Cell RNAseq Analysis Workflow



Differential Abundance

Aim:

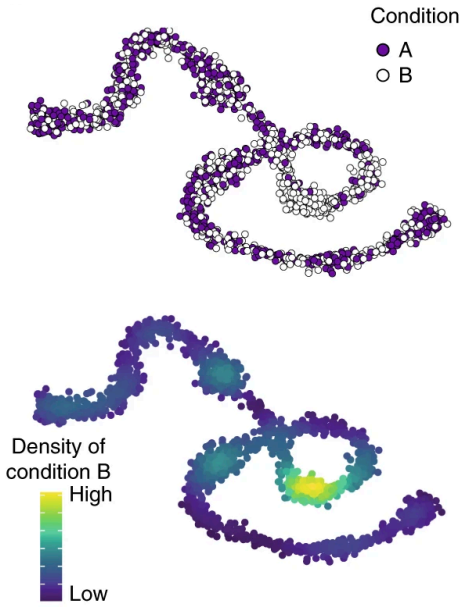
- test for significant changes in grouped cell abundance across conditions

Example:

- which cell types are depleted or enriched upon treatment?

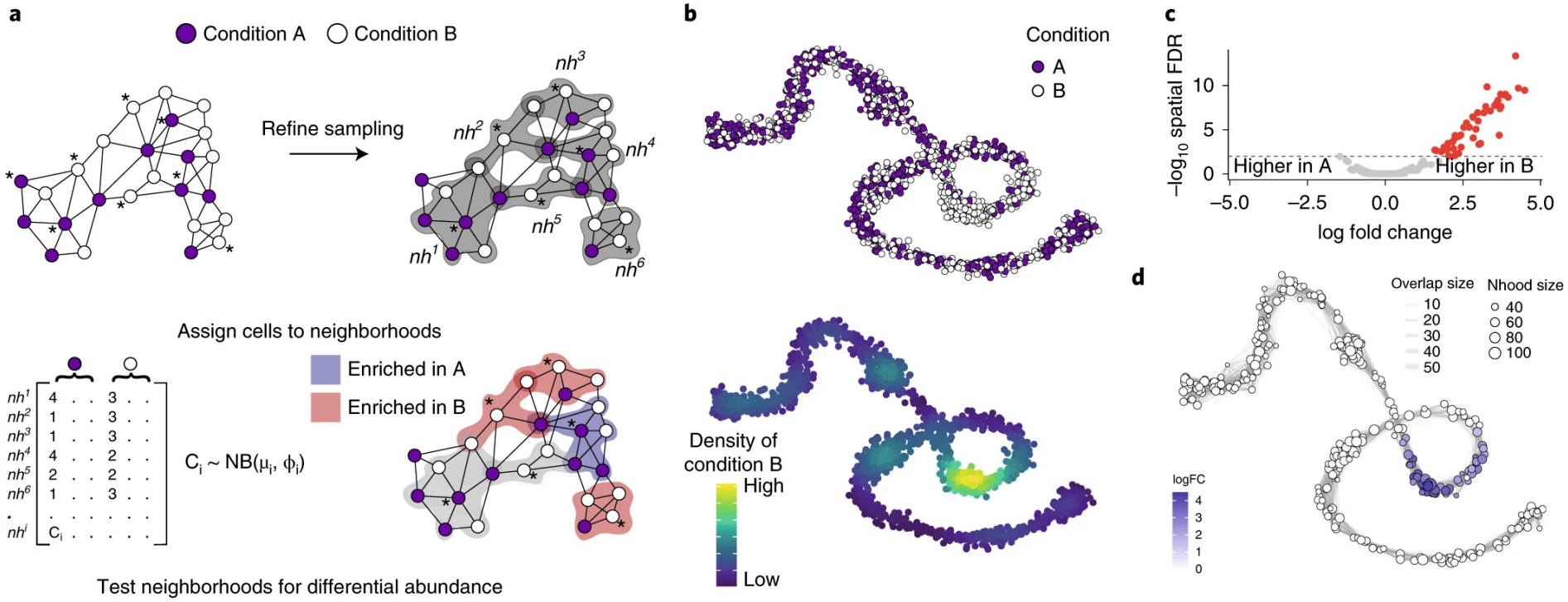
Differential Abundance - Milo

- Most methods require defined clusters as input. Assigning cells to discrete clusters in context of continuous differentiation, developmental or stimulation trajectories.
- Methods that don't require clusters also don't model variability in cell numbers among replicates or can only carry out pairwise comparisons.



- Can be used for complex experimental designs

Differential Abundance - Milo

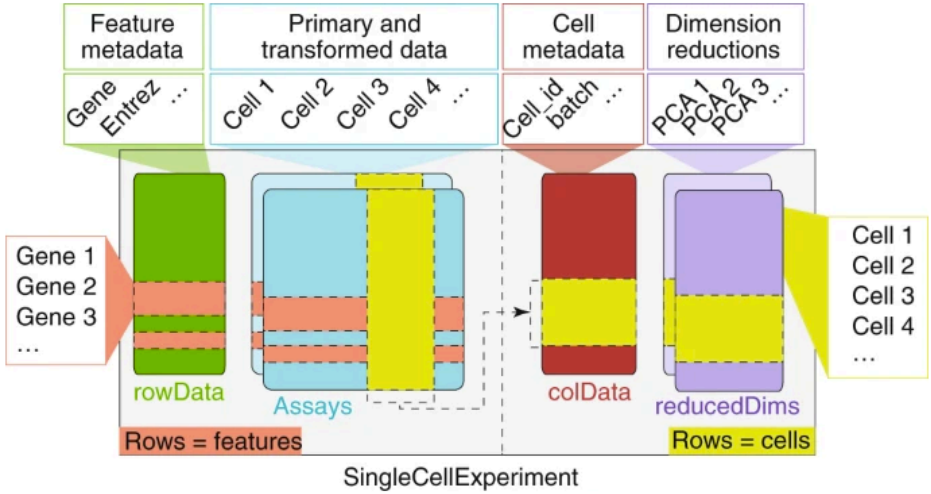


Differential Abundance - Milo

Steps:

- Construct KNN graph
  - rescales UMI count by per-cell sequencing depth
  - log transforms
  - uses PCA
  - calculates Euclidean distance between cells and its k nearest neighbour in PC space
- Defines Cell Neighbourhoods by sub-sampling the graph to identify useful “index cells” (for computational efficiency)
- Counts cells in Neighbourhoods
- Tests for DA in Neighbourhoods
- Does a multiple testing correction (Spacial FDR)
- Visualises the outputs with our UMAP embeddings

Conversion to SingleCellExperiment



- Milo is implemented in R and uses the SingleCellExperiment class as input
- We will have to convert our **Seurat** object to a **SingleCellExperiment** object
- Seurat has a function for this called `as.SingleCellExperiment()`
- The SCE object also has slots for the differnt elements of our data
  - `colData` for the metadata
  - `assays` for the count data
  - `reducedDims` for the dimensionality reduction embeddings