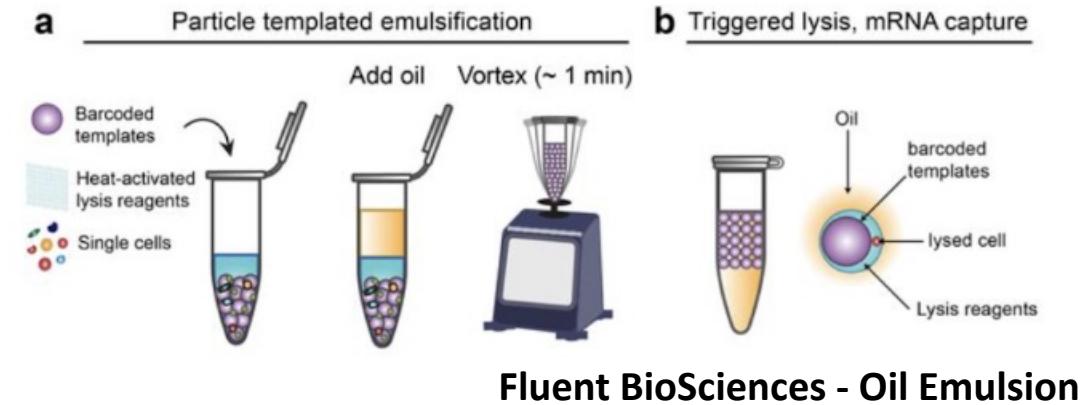
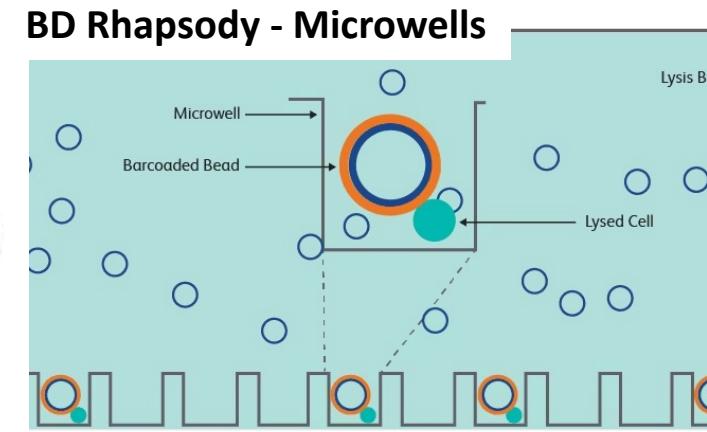
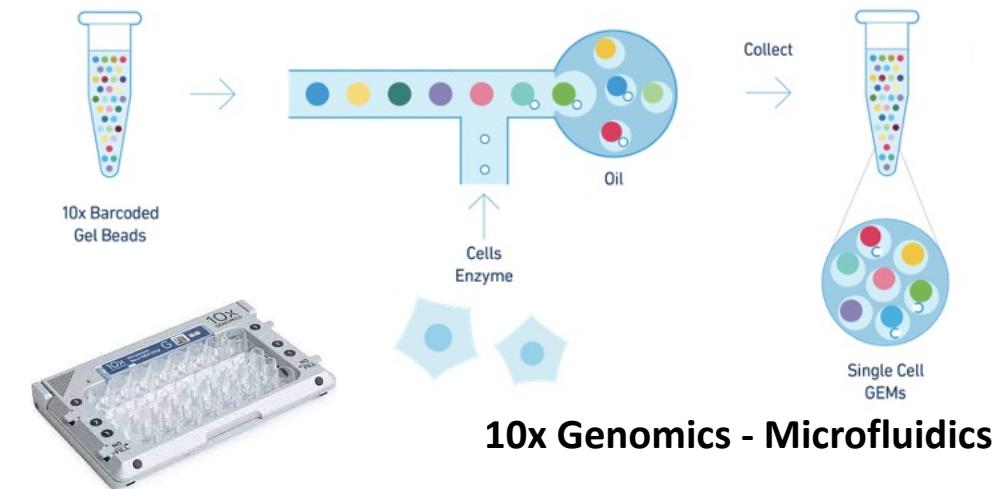
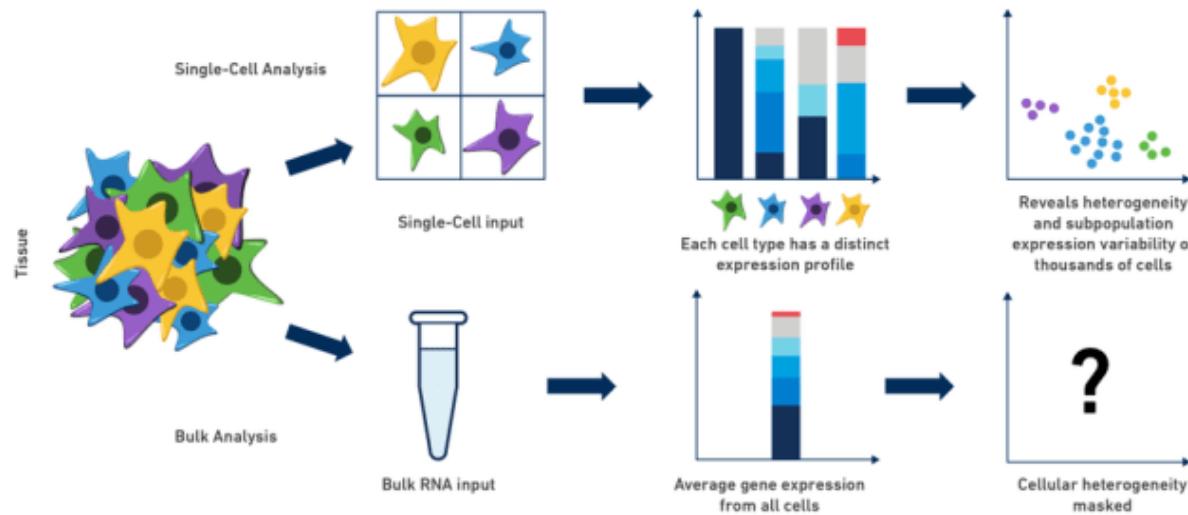
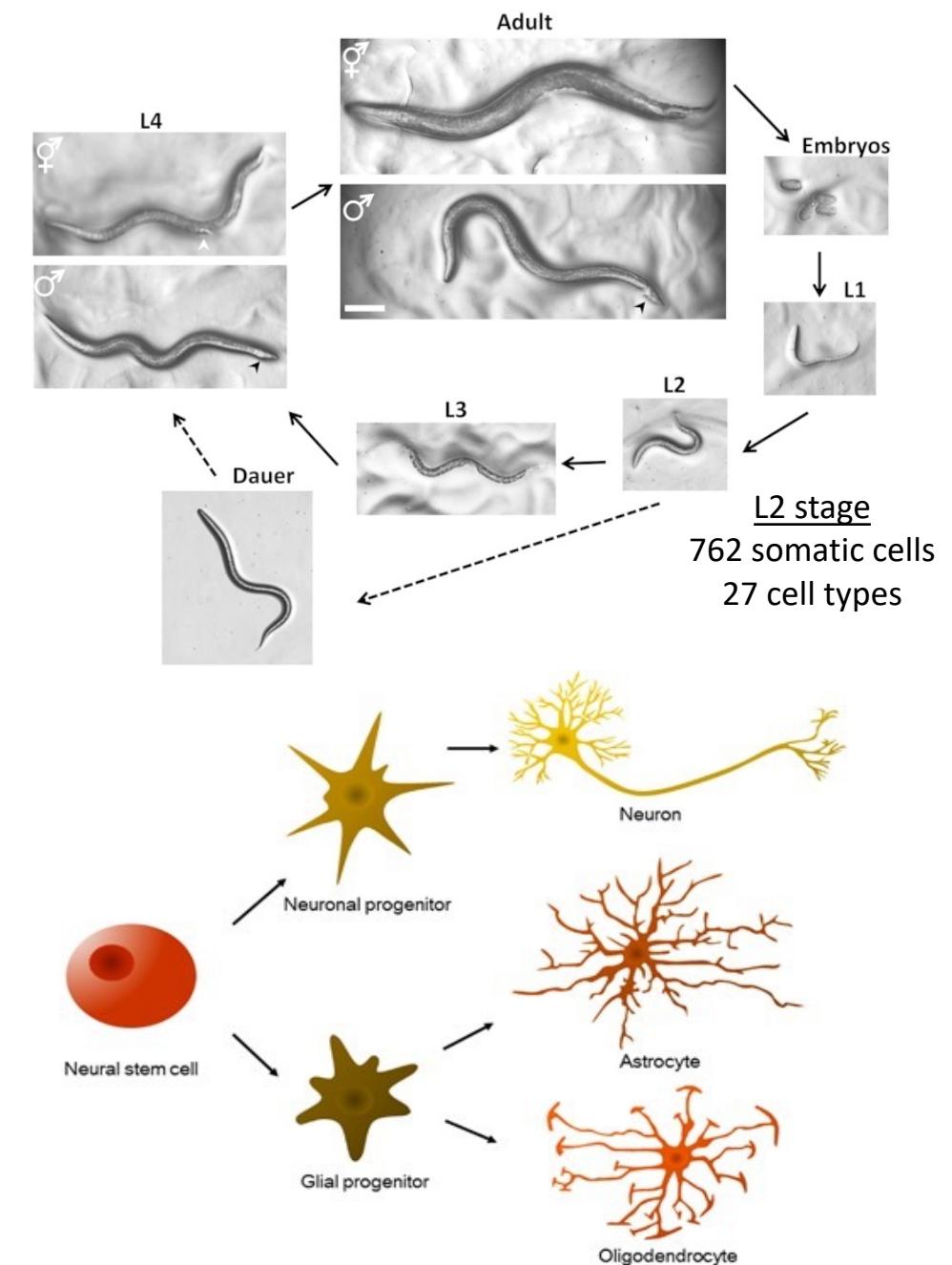


Introduction to single cell RNA-Seq for trajectory analysis

Single Cell RNA-Seq



- Suitable for
- High complexity input sample
 - Organs, whole organism, tissue, organoids, etc.
 - Unformal / Asynchronized cellular response / change
- Morphologically indistinguishable/ambiguous, or lack of markers
 - (Immuno-)Histology or flow cytometry
 - Cell type / state that are not previously defined
 - Non-model organism
- Rare cells / cell types
 - E.g, 1-2 rare neurons in L2 stage *C. elegans*
- Related cell types
 - Cell differentiation
 - Neuronal cell
 - Hematopoietic Stem cell
 - Activation
 - Adaptive immune response

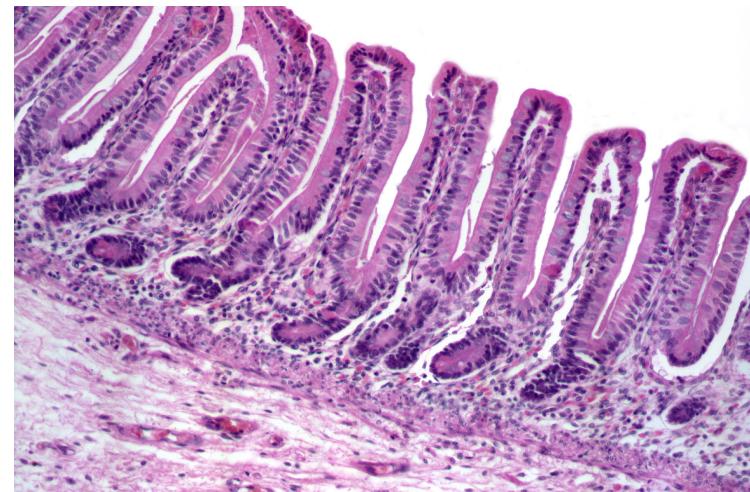
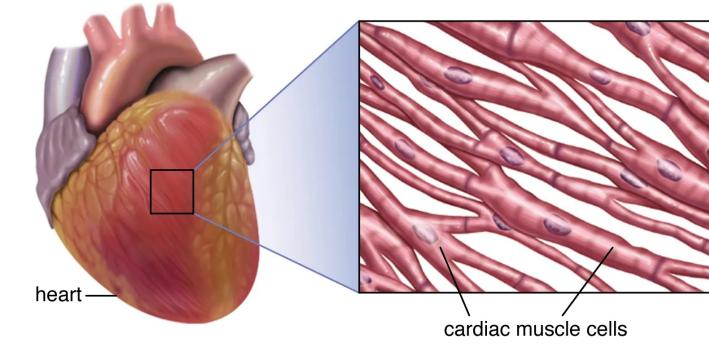
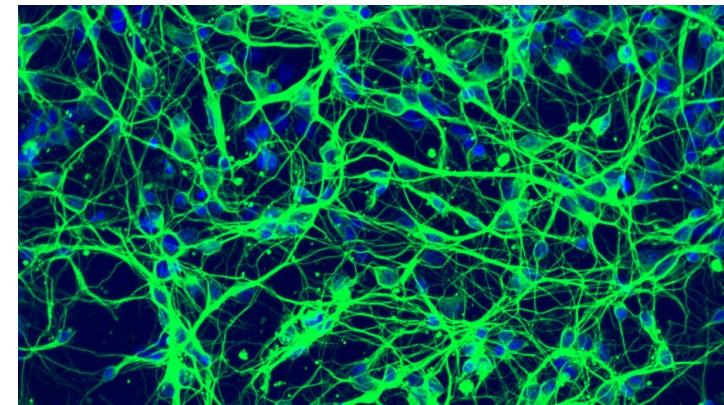


https://www.ncbi.nlm.nih.gov/books/NBK299460/figure/celegansintro_figure2/

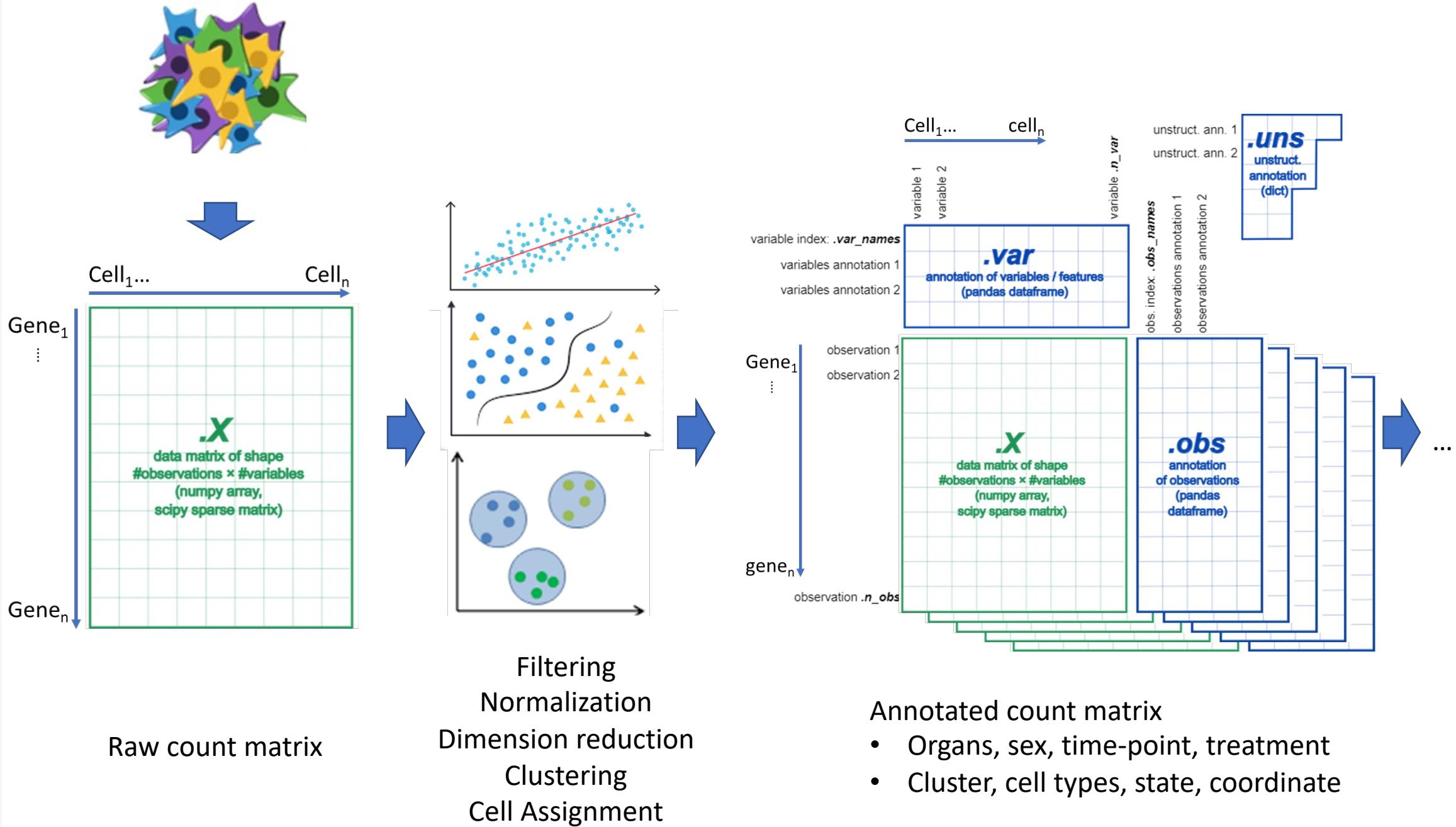
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Single cell RNA-Seq

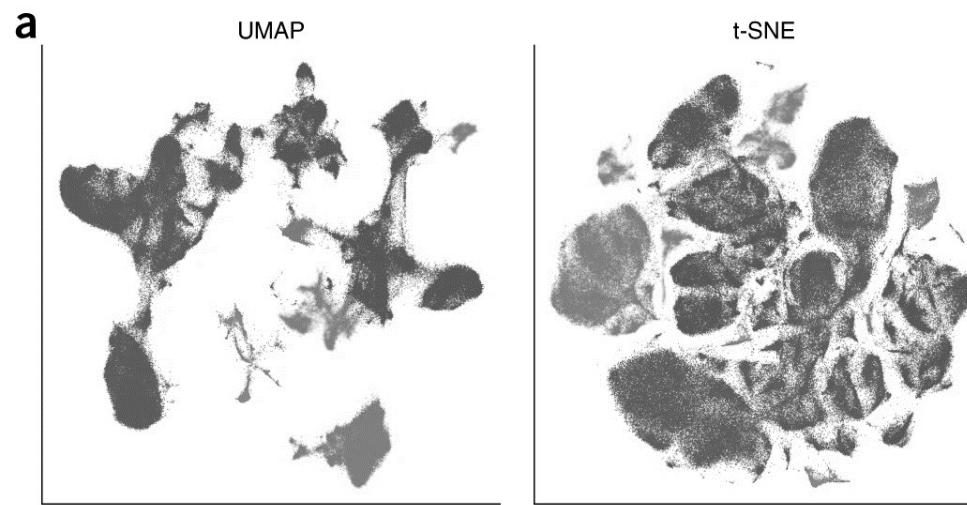
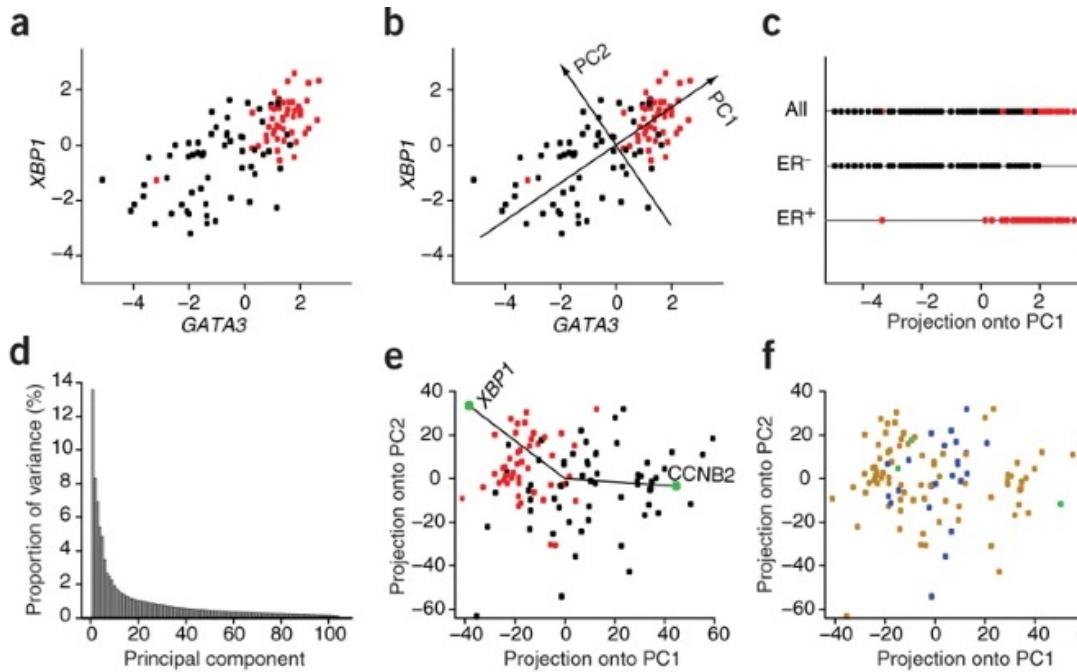
- Limitation
 - Costly
 - Instrument, reagents & sequencing
 - Incomplete transcriptome
 - Only mRNA transcripts that are highly expressed
 - Non-coding or small RNAs are omitted
 - Lack of isoform / splice variants information
 - Partial vs full transcript
 - mRNA transcript level does not always correlate with protein level
 - Expression of known protein markers may not reflect in transcript data
 - Single cell suspension required
 - Dead cell, damaged cell, nuclei only, cell fragment, incomplete / biased dissociation
 - Single nuclei as compromise for “difficult tissue”
 - Complexity of data analyses / interpretation
 - Quality control, e.g., live/dead, fragment, multiple, etc
 - Cell classification, e.g., Unknown, subtype, non-model organism, etc
 - Lack of spatial correlation



Data structure



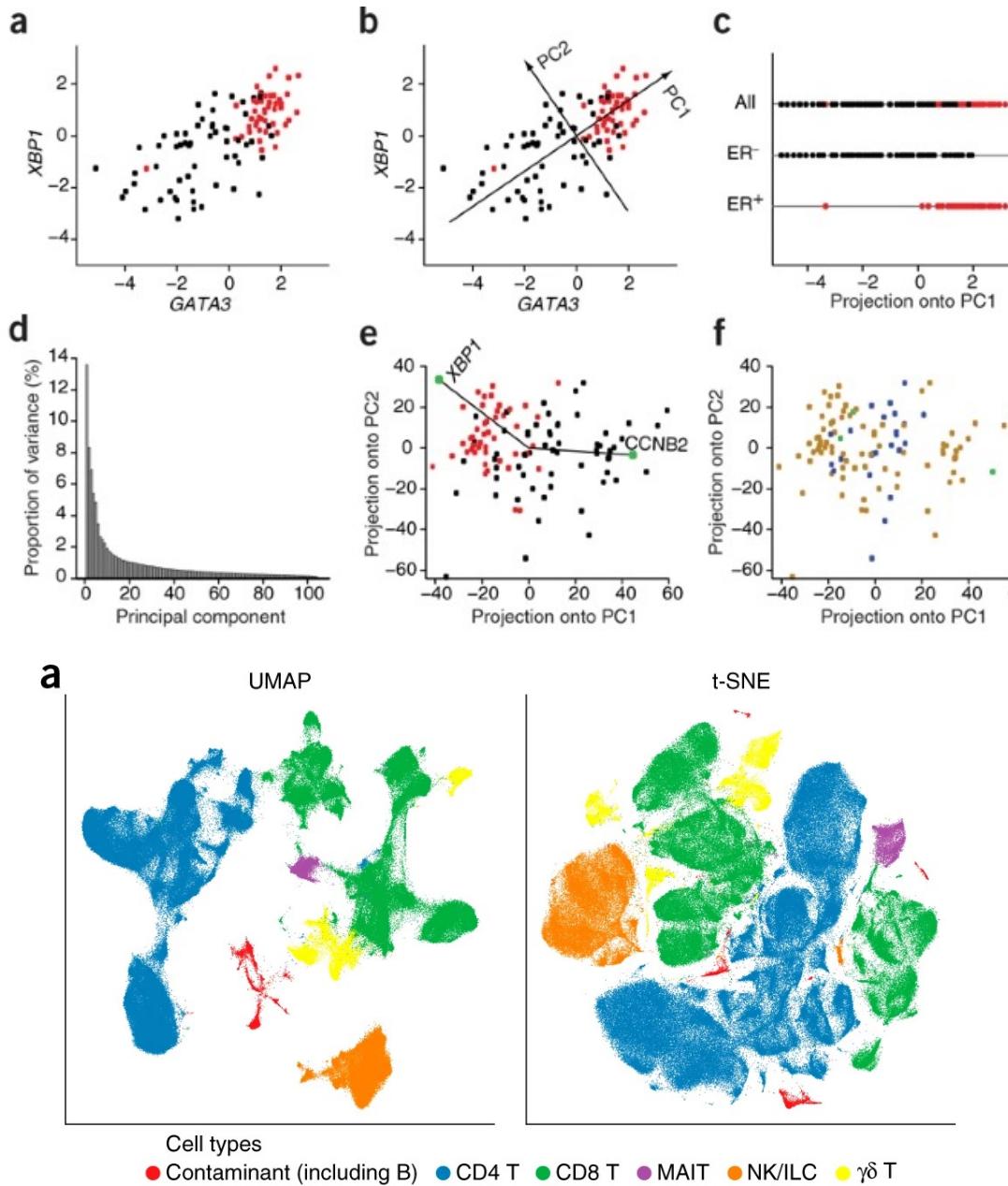
Dimension reduction, clustering, cell assignment



Uniform Manifold Approximation and Projection &
t-distributed stochastic neighbor embedding

<https://www.nature.com/articles/nbt0308-303>
<https://www.nature.com/articles/nbt.4314>
<https://www.nature.com/articles/s41598-020-59827-1>

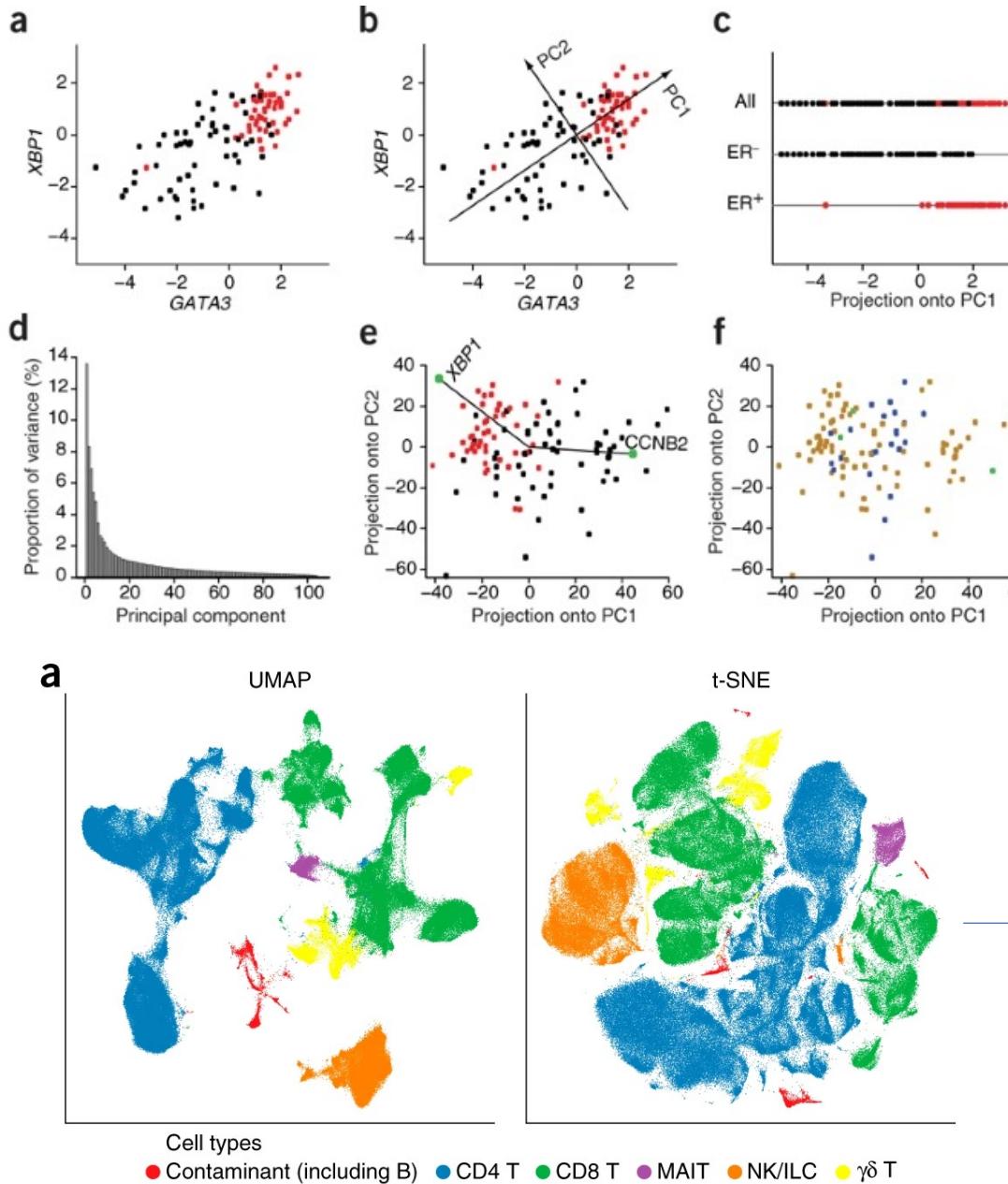
Dimension reduction, clustering, cell assignment



Uniform Manifold Approximation and Projection &
t-distributed stochastic neighbor embedding

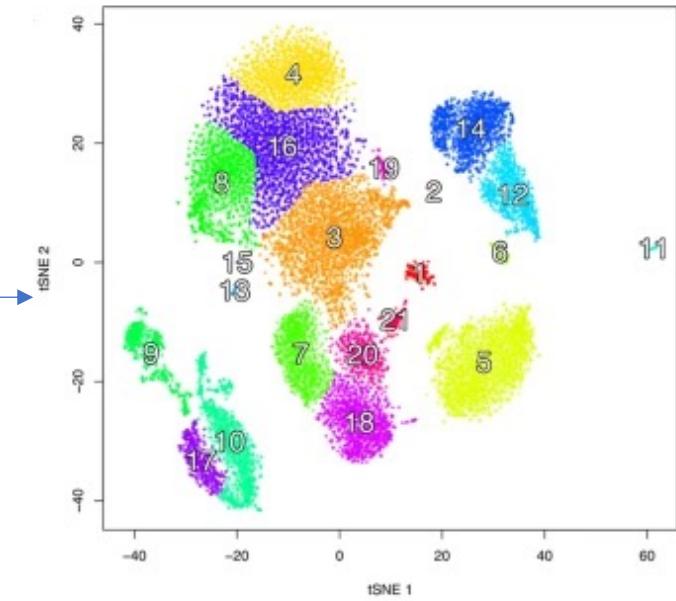
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Dimension reduction, clustering, cell assignment



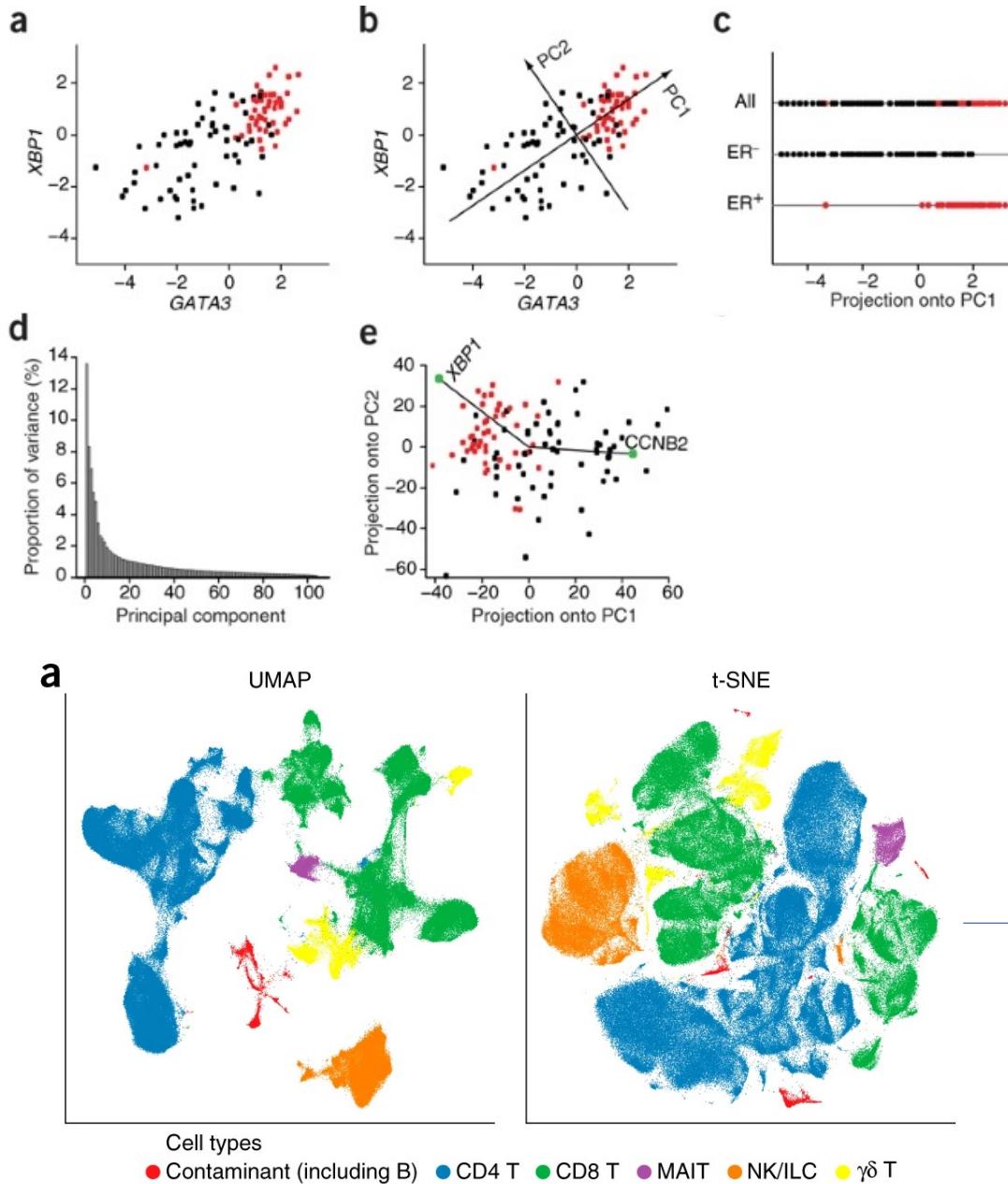
Uniform Manifold Approximation and Projection &
t-distributed stochastic neighbor embedding

K-nearest neighbor (KNN) graph

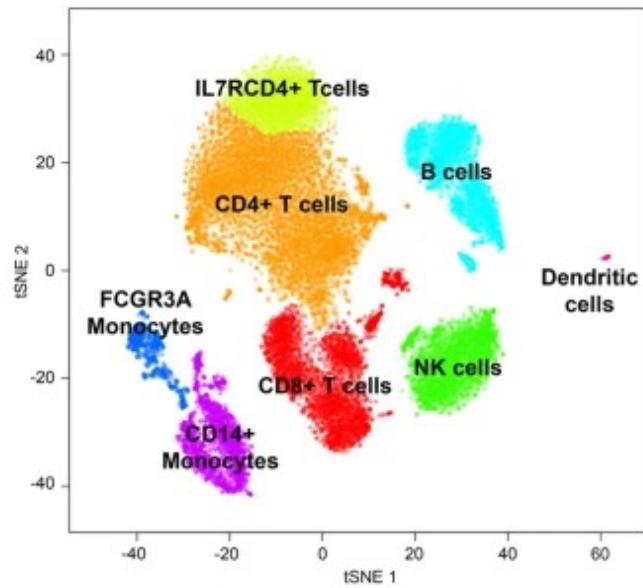
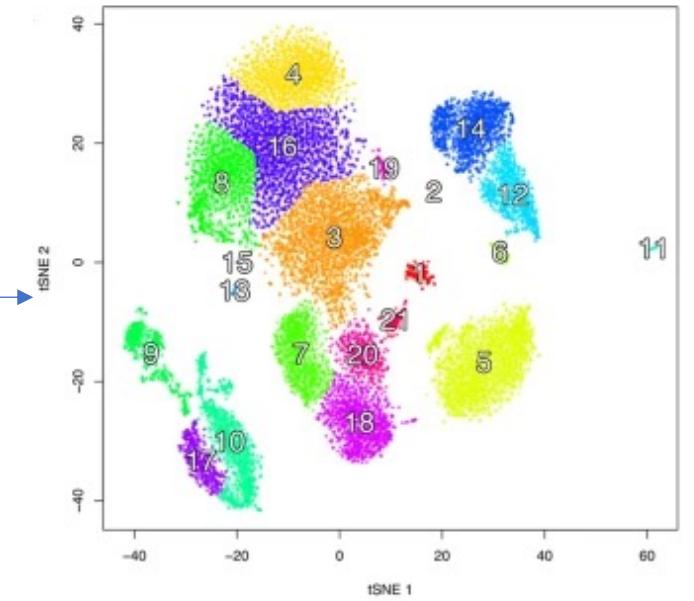


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Dimension reduction, clustering, cell assignment



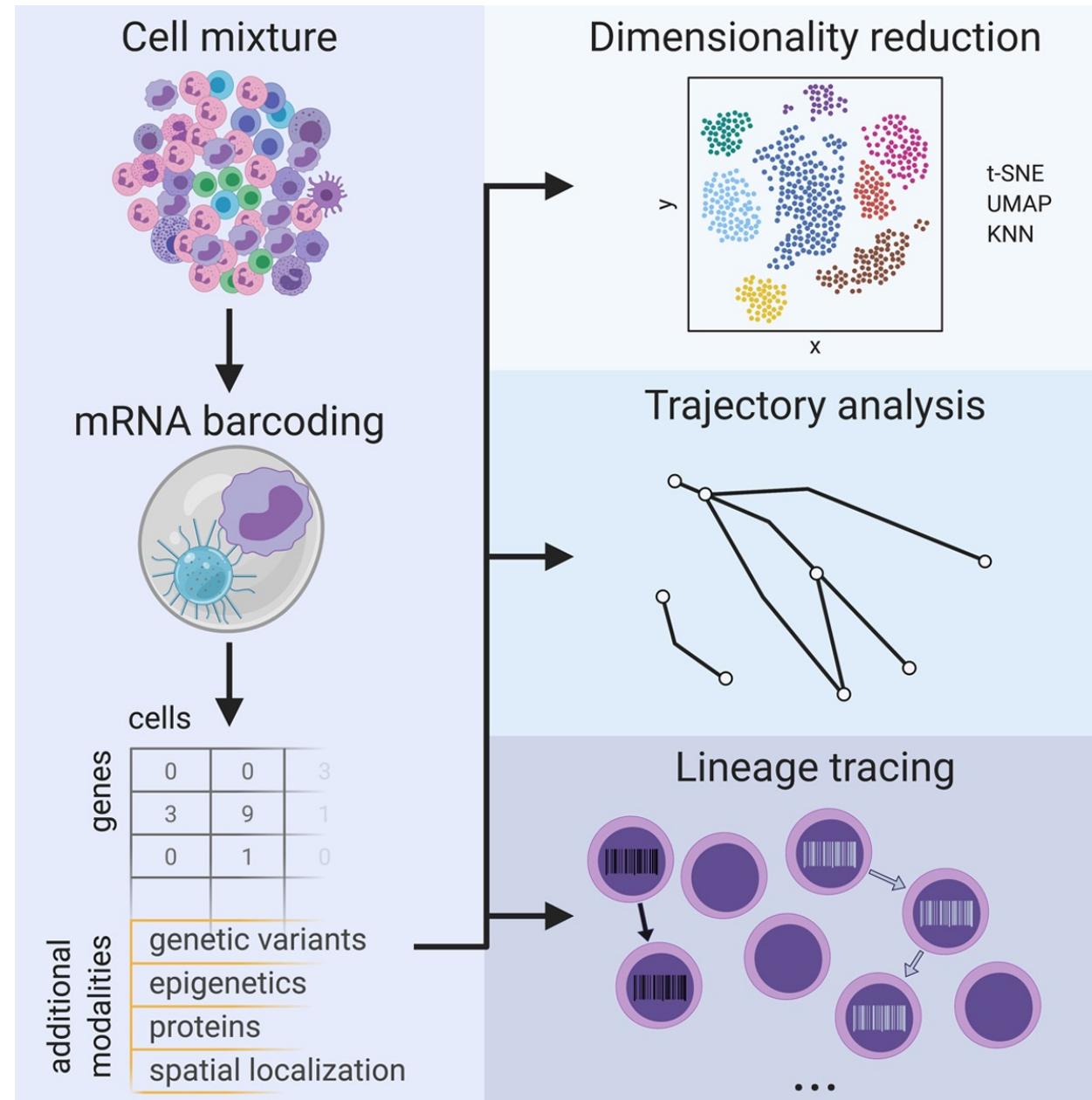
K-nearest neighbor (KNN) graph



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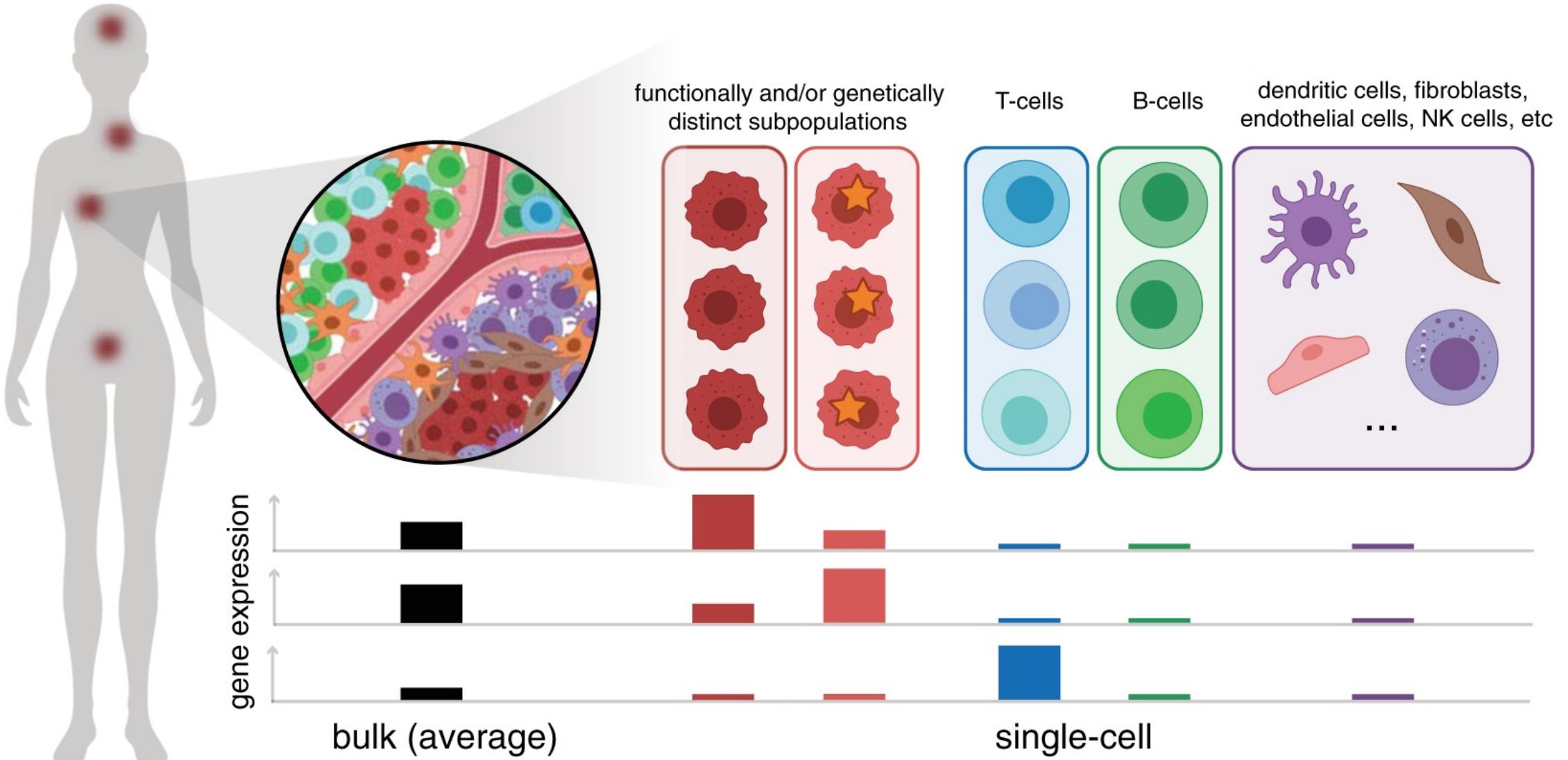
<https://www.nature.com/articles/nbt0308-303>
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What's next?

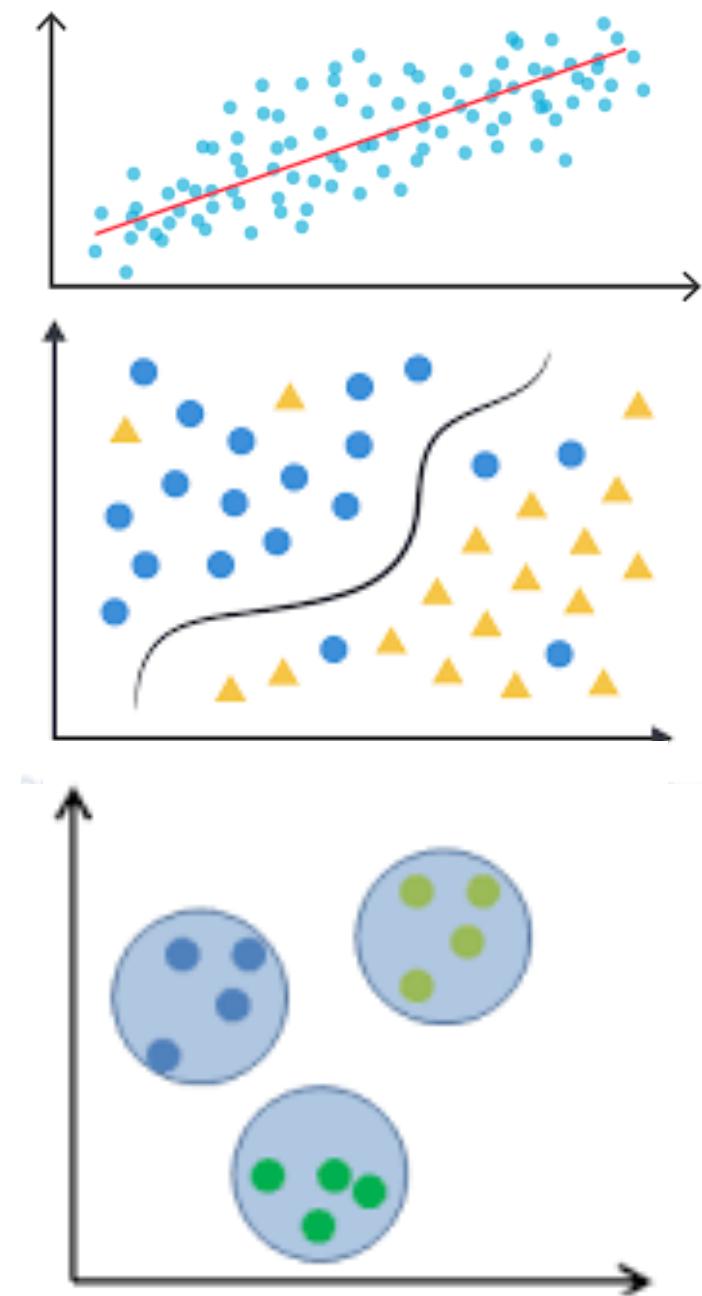
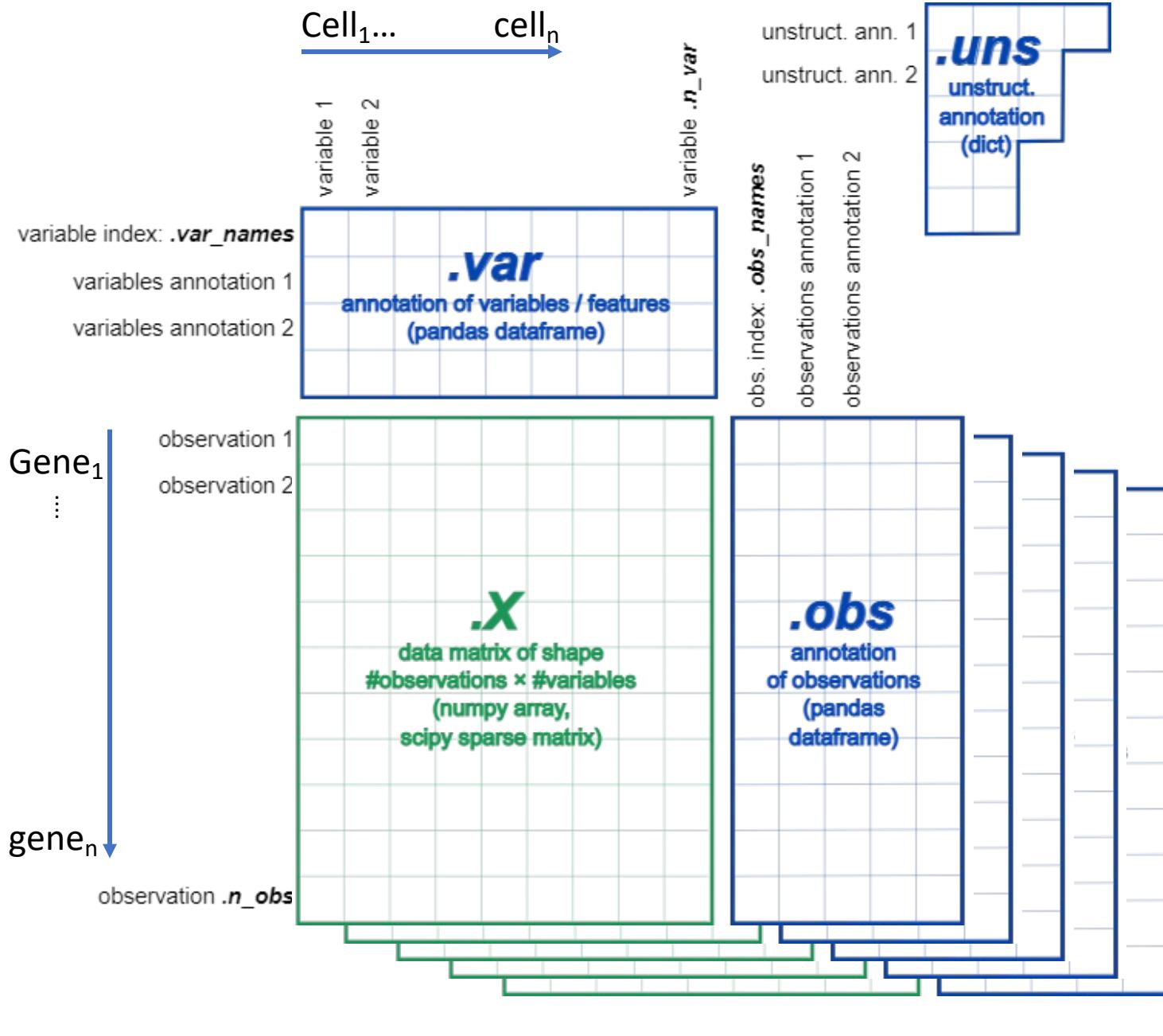


Extra slides

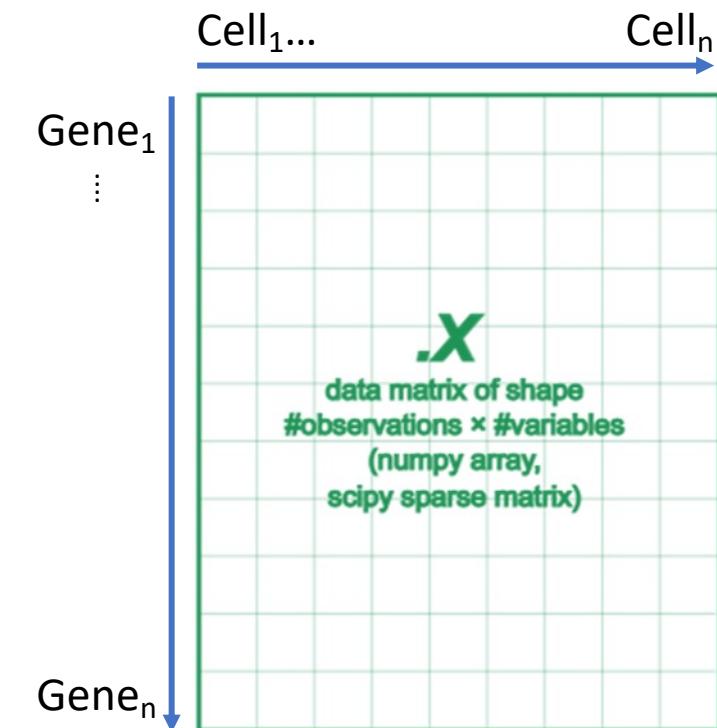
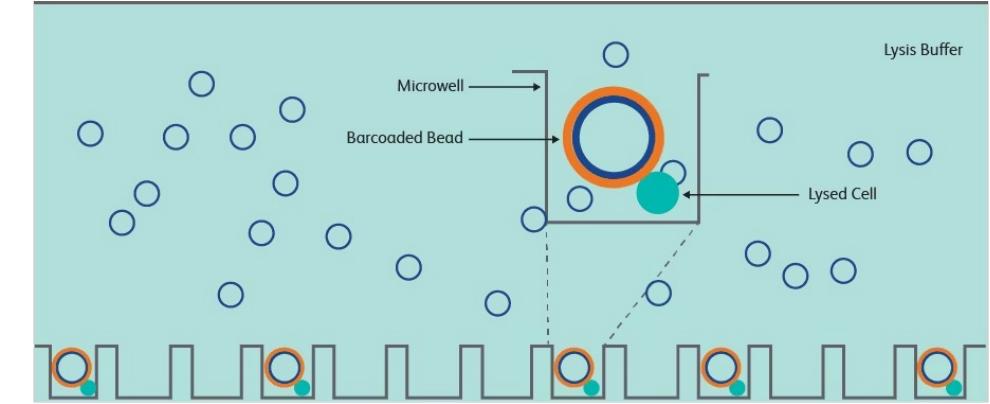
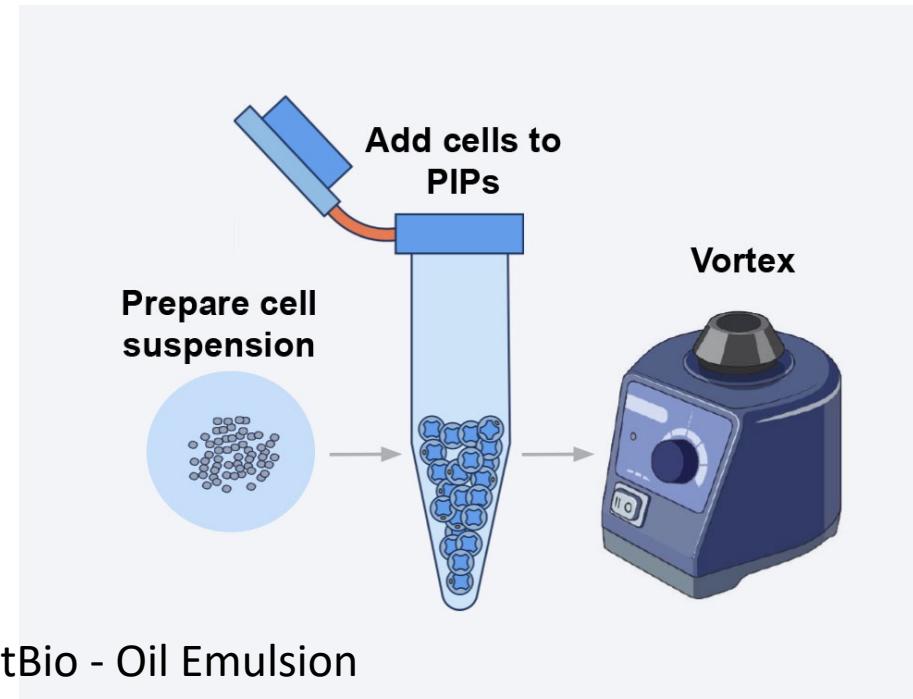
Single cell RNA-Seq



Data structure



Single cell RNA-Seq via Compartmentalization



Single Cell RNASeq

- A power technology that allows to acquire transcriptome profile within a single cell
- Typically, 1000 – 10,000 cells per conditions, up to million of cells in a single experiment
- Cells can be grouped and defined based on transcriptome profile, follow by group specific differential expression analysis
- Multiple technologies, varying in
 - Throughput
 - Sequencing / detection format & requirement
- High complexity input sample
 - Organs, whole organism, tissue
- Morphologically identical similar or lack of markers
 - (Immuno-)Histology or flow cytometry
 - Cell type / state that are not previously defined
 - Non-model organism
- Rare cells
- Costly
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