

Transcriptomics Part 3: scRNA-seq

Bridging the Bench-Machine Learning Gap

Dr. Emily A. Beck

Dr. Jake Searcy

Learning Objectives

Understand the biology behind single cell sequencing

Think critically about when to employ these techniques and the pros and cons to consider

Why is scRNA-seq so popular?

Single cell RNA-seq is useful for determining how different cell types' expression patterns are impacted.

If you are working with a tissue of multiple cell types (most tissues) this is helpful for determining expression changes in less abundant cell types

Plate-based SMART seq

General pipeline: use flow cytometer to sort cells/lyse cells/library prep

(1) Reverse transcriptase build off a off PolyA start site to make cDNA

NNNNNNNNNNAAAAAA

(2) Polymerase add non-template Cs at the end

NNNNNNNNNNAAAAA
CCCCNNNNNNNNNN

(3)Template switch oligo with G overhang

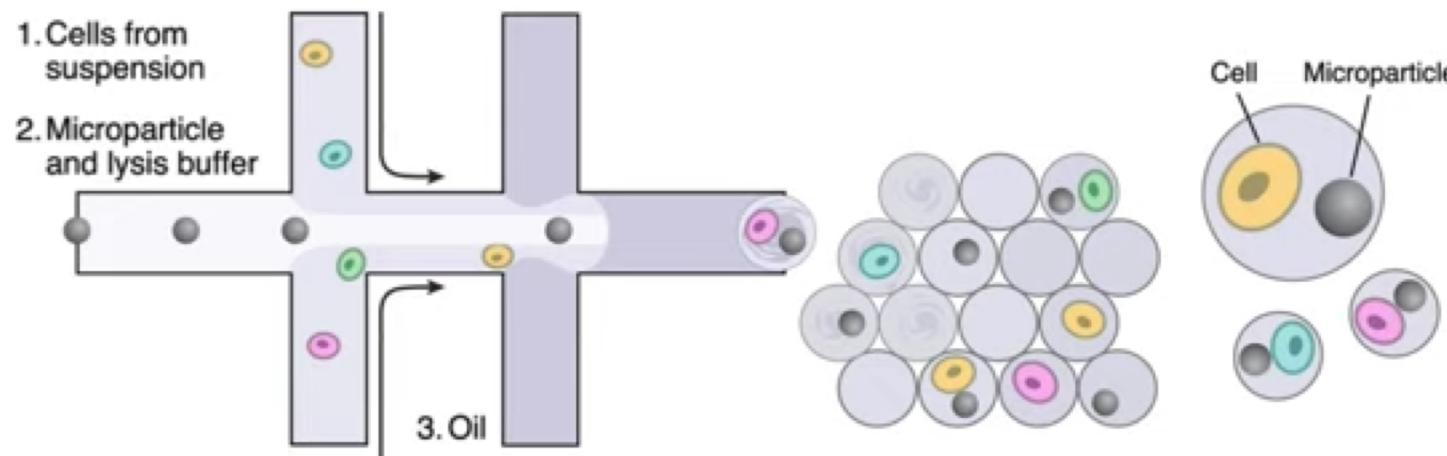
←
GGGNNNNNNNNNNAAA
CCCCNNNNNNNNNN

END: cDNA with PCR "handles" on both ends then amplify to make copies of each cDNA fragment. One cell per tube (Labor intensive)

Drop-Seq (Parallelizing the Plate-based method)

Use microfluidic chip

Oil droplets are used to combine individual cells and
Barcoded beads or particles into single droplets



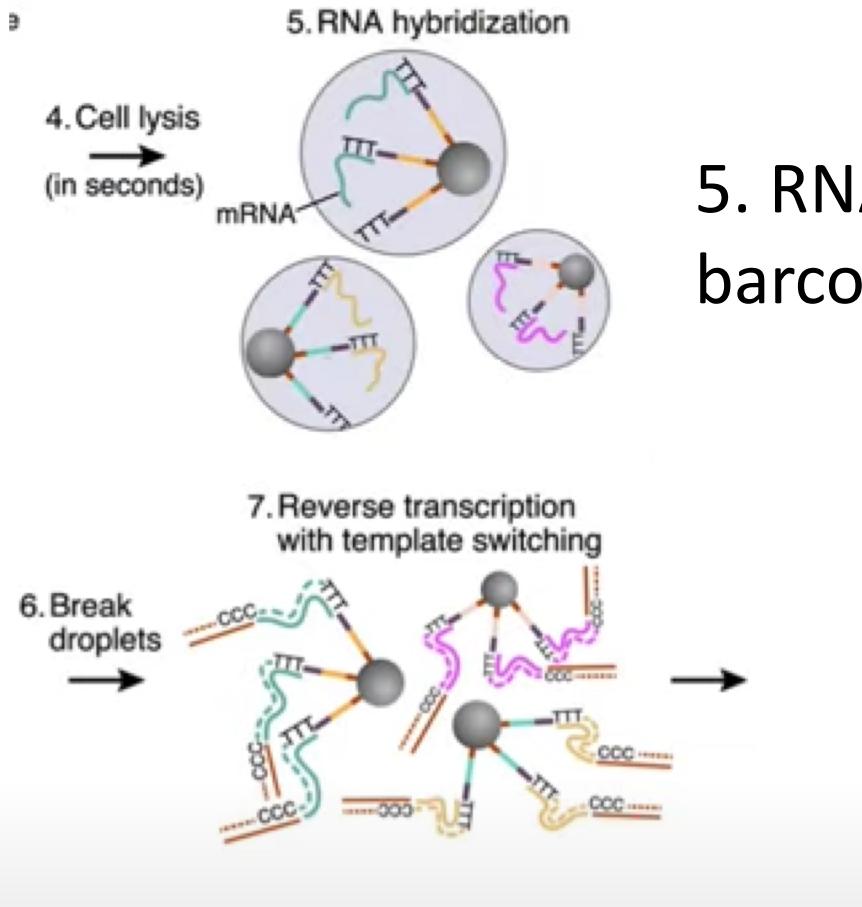
Small fraction end up
with BOTH a cell and a
barcoded microparticle

Not shown but another problem is multiple beads ending up in a droplet (doublets)

(Macosko et al. 2015)

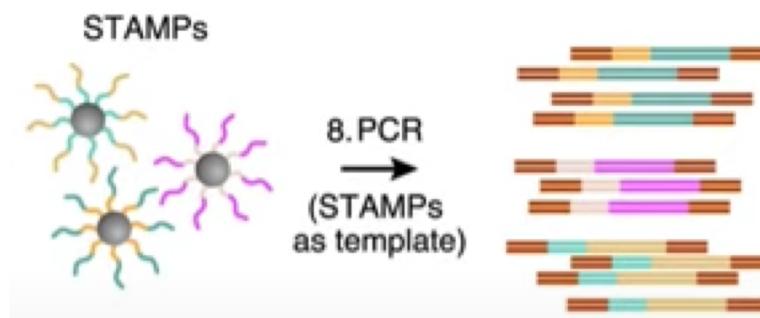
Drop-Seq (Parallelizing the Plate-based method)

Select for droplets with cells and beads and lyse the cells



5. RNA from the cells then hybridizes to the unique barcodes on the beads

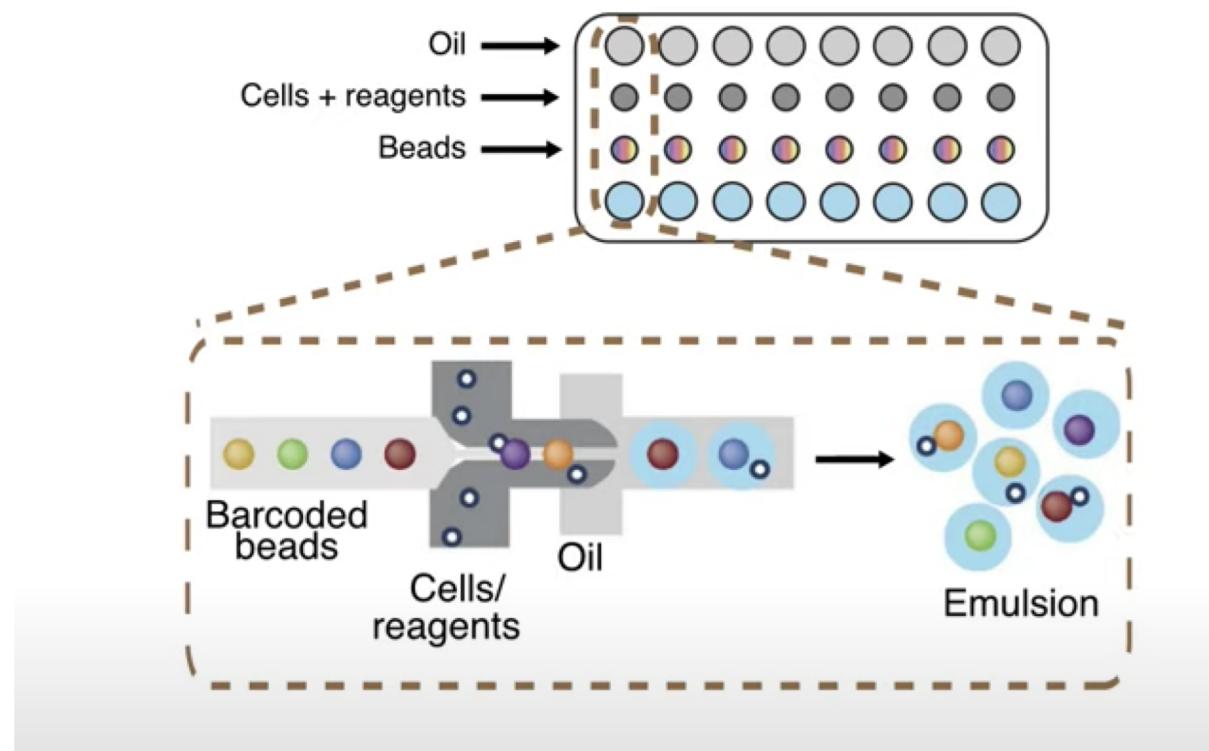
7. Following the same oligo switching method you build cDNA



STAMPs
Single-cell Transcriptomics
Attached to Microparticles

(Macosko et al. 2015)

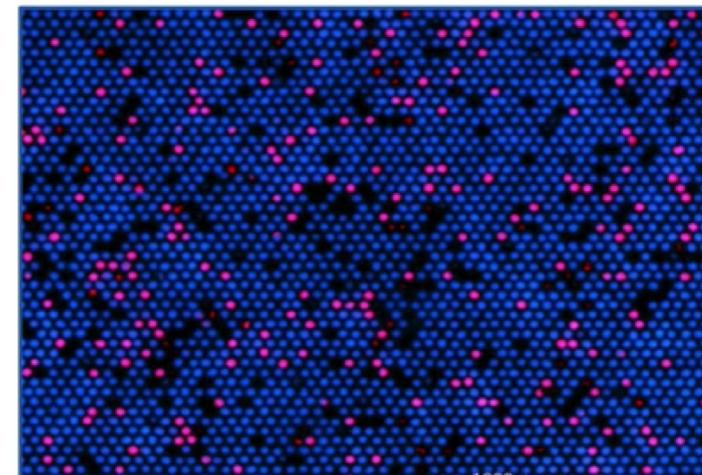
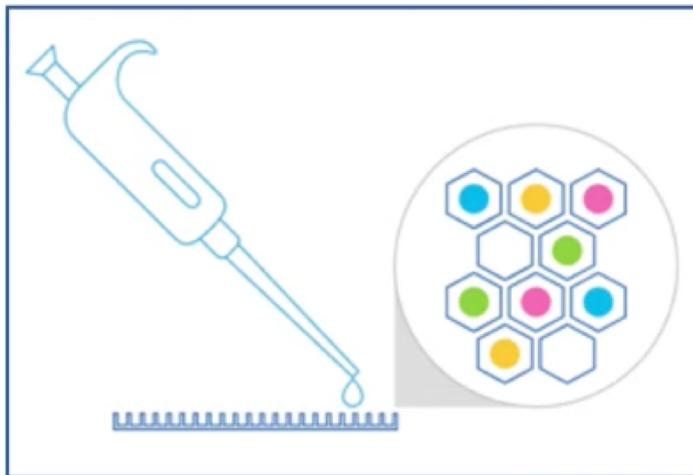
10X Genomics modified further



Engineered their chip and beads so that 90% of emulsions contain only one bead.

Microwells- parallelized without droplets

Microarray of wells small enough for a single barcoded bead to enter single cells



Combinatorial Indexing (*in situ* method for barcoding)

Massively increase number of cells in one run

Through a series of mixing and pooling you generate combinatorial barcodes to generate single cell data

(1) Start with a plate with individual barcodes in each well

(2) Add cells

(3) Cells are randomly assigned to one of the wells

Barcode Combinations				
1	96	384	1536	
96	9,216	36,864	147,456	
384	36,864	147,456	589,824	
1536	147,456	589,824	2,359,296	

(4) Pool cells and barcode a second time on another plate

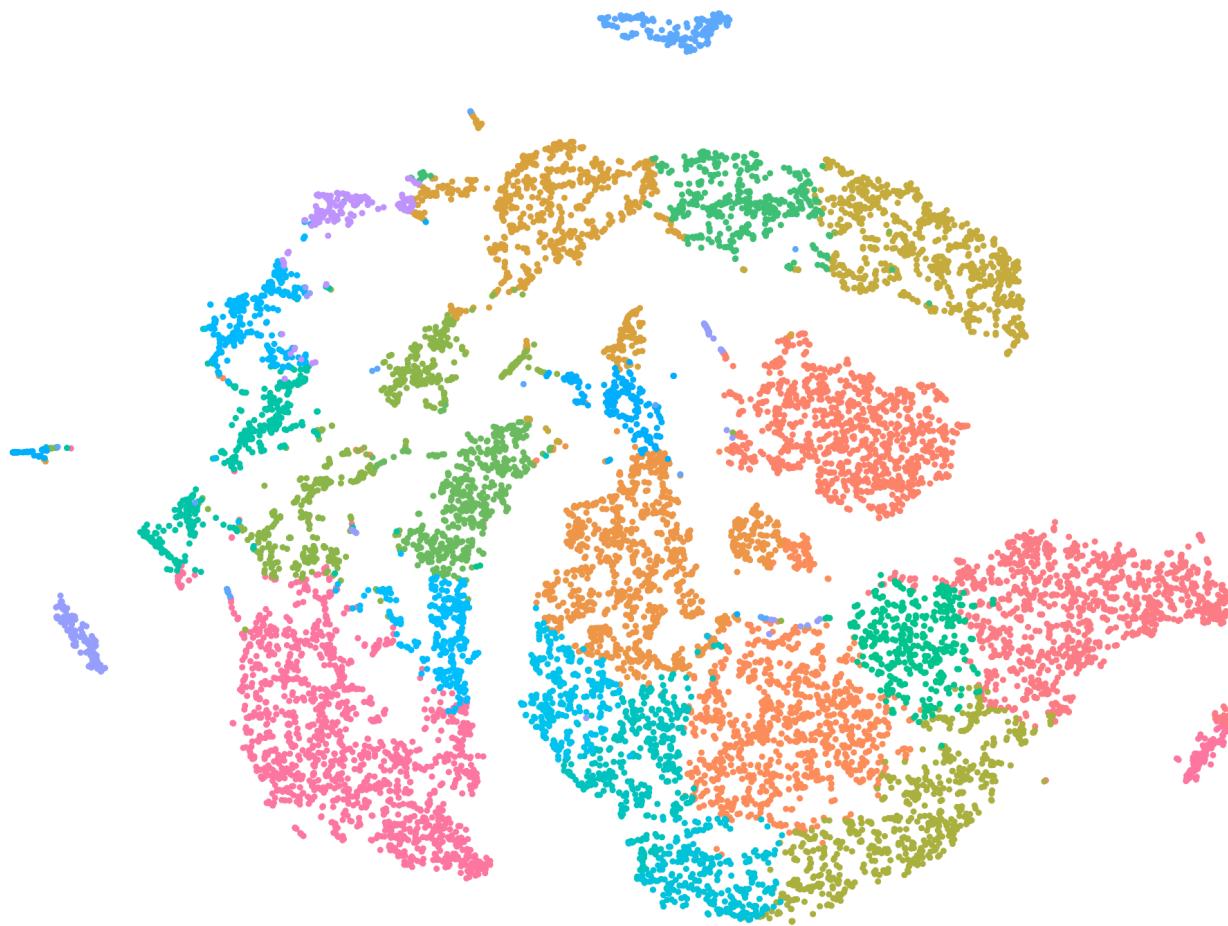
This sounds great but there are challenges to single cell RNA-seq you should consider

Major Problem #1: Low % capture of the transcriptome

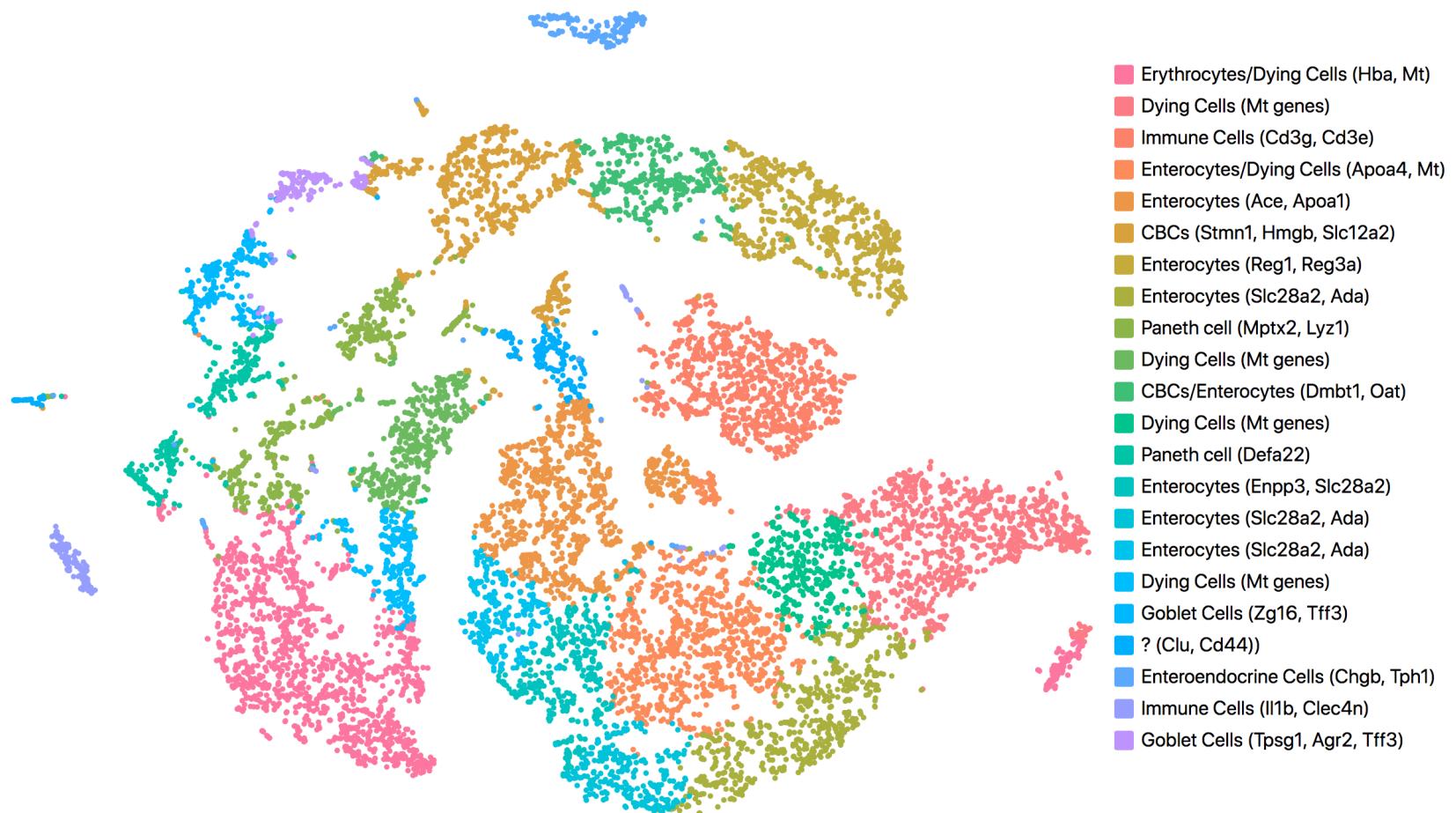
The latest version captures at best 30-40% of the transcriptome.
This is a huge problem if you are comparing across treatments
or are interested in rare variants

Major Problem #2: Lack of cell atlases for non-models

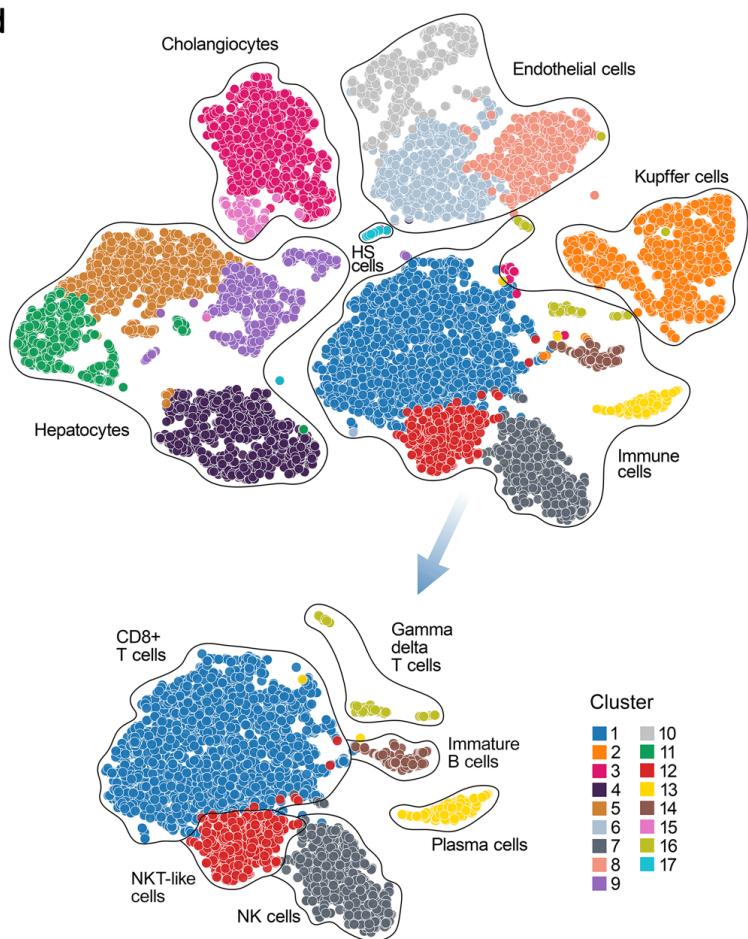
Clusters like this are pretty but not useful without an atlas



We will talk later about going from reads to counts to clusters, but once you are at clusters an atlas is needed to make biological inferences



You can also ask interesting questions about cell type heterogeneity using scRNA-seq and sn-RNaseq



After initial clustering you can focus on a specific group and cluster further to look for smaller groups within your identified cells