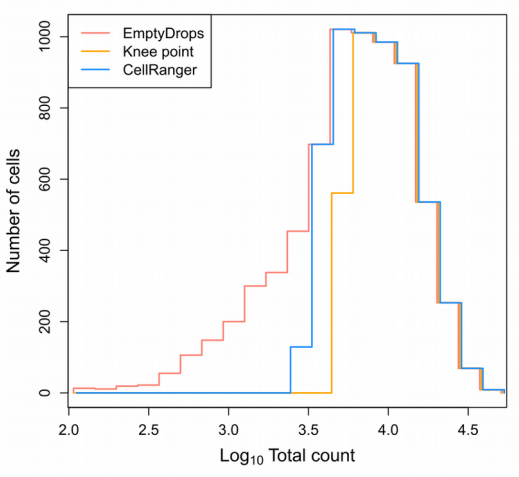
Single Cell Transcriptomics

Newsletter April 2019

Paper 1

Lun, A. T. L. et al. [EmptyDrops: distinguishing cells from empty droplets in droplet-based single-cell RNA sequencing data] *Genome Biology*, 2019.

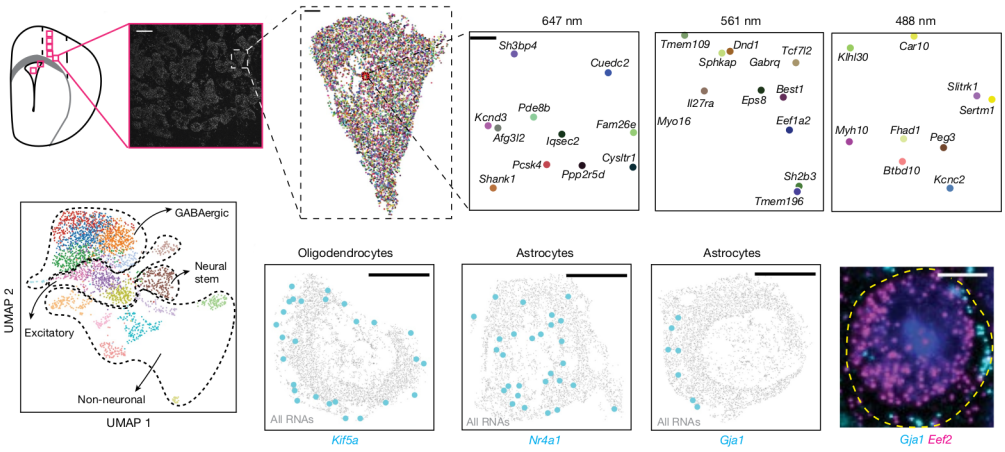
A new method for detecting empty droplets in droplet-based single-cell RNA sequencing data was developed. Standard approaches (including one implemented in cellranger) are based on removal of drops with lower-than-threshold total UMI count. Such approach also leads to unspecific removal of drops containing small cells/nuclei containing low RNA. New method is based on comparison of every drop to the estimated expression profile of ambient RNA (drops with the lowest UMI counts). Drops with significant deviations from the ambient RNA are considered to be genuine cells, thus allowing recovery of cells with low total RNA content and small total counts. This is combined with a standard UMI knee point filter to ensure that drops with large total counts are always retained. Based on simulations this method was shown to outperform previous approaches, especially in retaining small cells. Also, analysis of real datasets have shown that new method detects more cells and finds additional sub-populations (lymphocytes, small interneurons) discarded with previous approaches. Although, care needs to be taken in removing low quality cells based on other metrics (mtRNA, ribosomal protein mRNA, etc.).



Paper 2

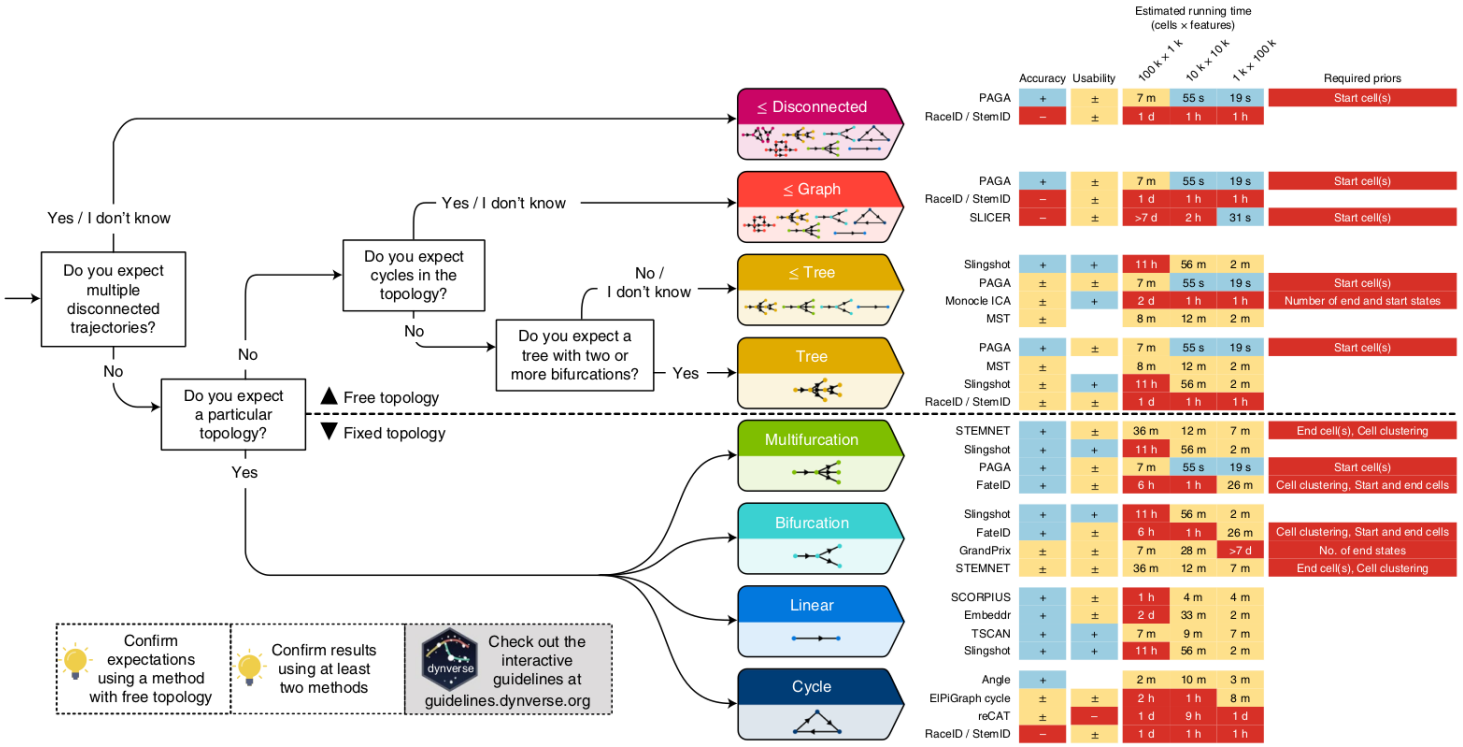
Eng, C. H. L. et al. [Transcriptome-scale super-resolved imaging in tissues by RNA seqFISH+] *Nature*, 2019.

A new method for spacial transcriptomics was developed. It is an improved successor of seqFISH method with increased resolution and throughput. seqFISH+ is based on the primary probe hybridization against RNA molecules. Readout of the primary probe identity (thus RNA identity) is based on the fluorescent signal from the array of secondary probes that hybridize to the overhangs on the primary probes. Fluorescent signal is detected using confocal microscopy. seqFISH+ enables identification of subcellular localization of 35,492 transcripts per cell when applied on cell samples. Cell RNA expression data produced with seqFISH+ correlates well with RNA-seq and smFISH data, and has detection efficiency of 49%, that is much higher than with scRNA-seq. SeqFISH+ can also be applied on tissue section. It detects 5,615 transcripts from **3,338 genes per cell** on brain sections, and this value could be further improved 5-10 times if imaging in multiple optical planes. seqFISH+ provides >10x improvement over existing methods in the number of mRNAs profiled per cell. Thus, this method is narrowing the gap between *in situ* and cell/nuclei based *in vitro* single cell transcriptome analysis approaches, while providing a valuable tissue localization information. Hovewer, the major consideration would be the amount of time needed for imaging the sections (only ~3000 cells analyzed on brain slices in current study).



Paper 3

Saelens, W. et al. [A comparison of single-cell trajectory inference methods](#) *Nat. Biotechnology*, 2019.



Dynamic cell processes, such as cell cycle, cell differentiation and cell activation, leave their fingerprints in the cellular transcriptome, epigenome and proteome. Already more than 70 tools have been developed to study these processes in the single cell data. Although, a high number of tools make selection of the best one a challenge. In this study 45 of trajectory inference (TI) methods were benchmarked on 110 real and 229 synthetic datasets for cellular ordering, topology, scalability and usability. Substantial complementarity between methods was found, with different sets of methods performing most optimally depending on the characteristics of the data. Thus, interactive set of guidelines was created (guidelines.dynverse.org), which gives context-specific recommendations for the most suitable method.

Next Single Cell Seminar

26th April 2019, Mærsk Tower, Top Floor

9:00 – 9:40

Mette Ludwig, CBMR - Single cell transcriptomics and epigenomics guided identification of GLP-1 responding cell types in the area postrema of the hindbrain

10:00 – 10:40

Kedar Nath Natarajan, BMB, SDU - Cell cycle dynamics in embryonic stem cells

10:40 – 11:00

Coffe and discussion

Meetings

April 11th, 2019 Single-cell user group meeting, SDU Odense

Trainings

Next Generation Sequencing Bioinformatics EMBL-EBI, Hinxton, UK. Deadline 28th June 2019

Whole Transcriptome Data Analysis EMBL, Heidelberg, Germany. Registration will open soon

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