

Intermediate presentation

Differential regulation

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- Brief (re)-introduction to the topic
- Data selection process
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(Re)-Introduction to the topic

- Abundance of spliced and unspliced mRNA methods allows studying cellular dynamical processes, such as cell differentiation, cell cycle and cell regulation
- Aim at discovering differential usage of spliced and unspliced counts between groups of samples in each cluster of cells

Data selection process

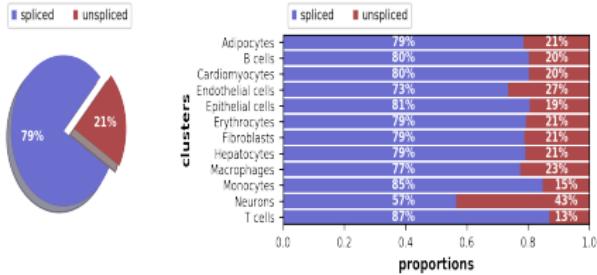
- Mouse data:
 - ▶ kidney cells from four biological replicates under the same experimental condition (Park et al. (2018))
- Human data:
 - ▶ 16 brain organoids (PGP1 cell line) from three different batches at three months and six months growth (different experimental condition) (Velasco et al. (2019))

Section 1

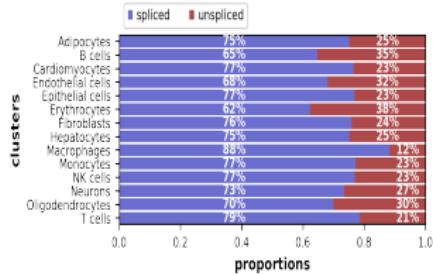
Mouse data

Looking at the proportion of spliced and unspliced counts

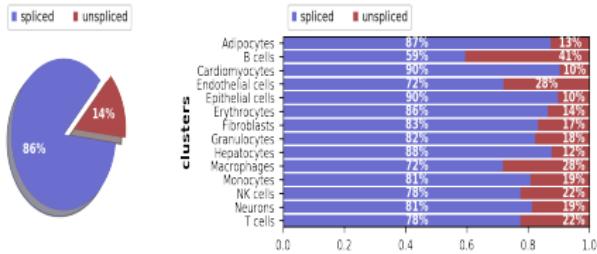
Normal 1



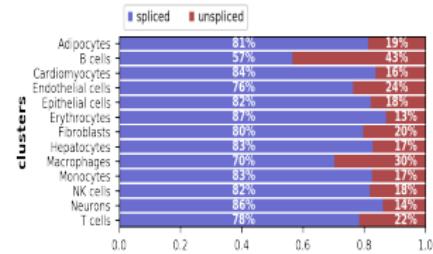
Normal 2



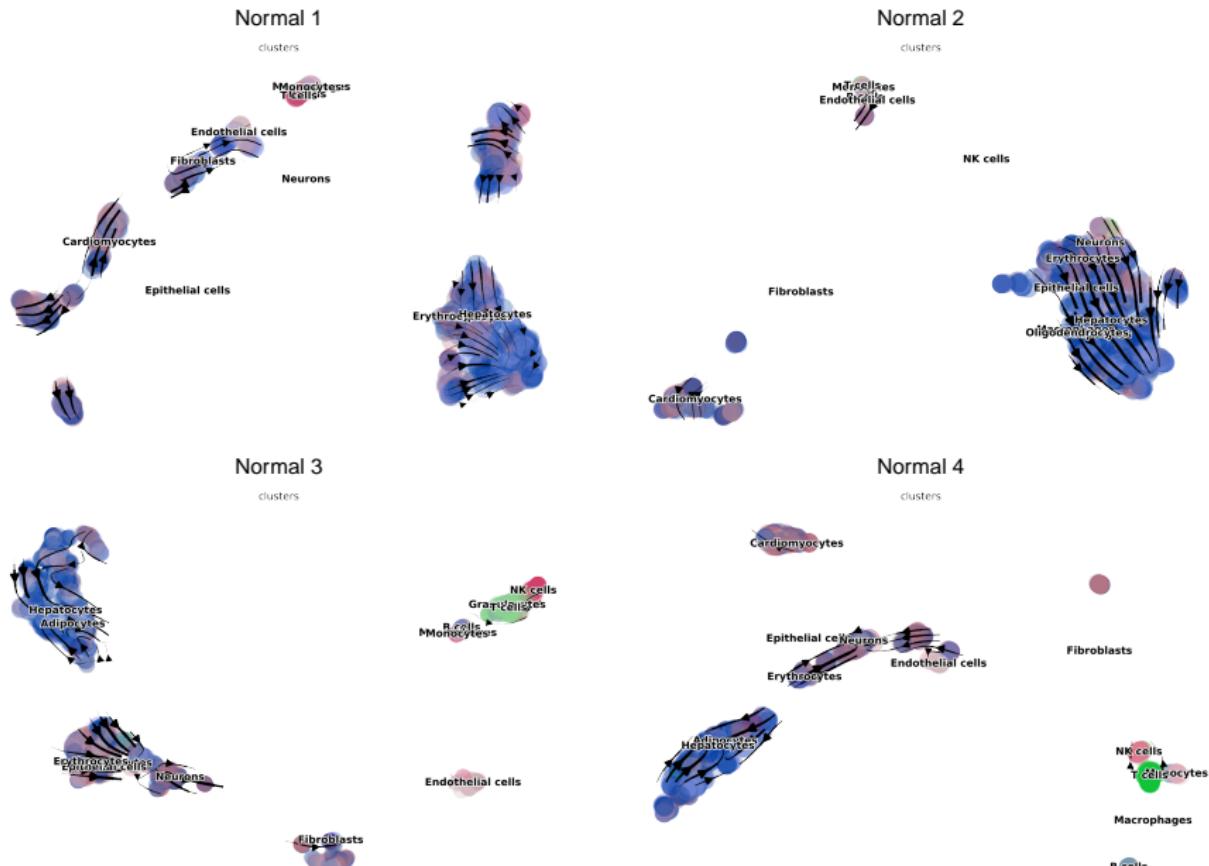
Normal 3



Normal 4



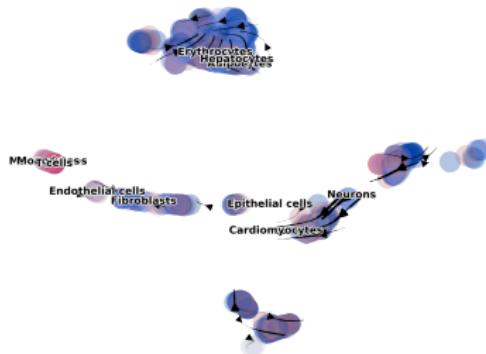
Looking at the RNA velocities - individual samples



Looking at the RNA velocities - all samples

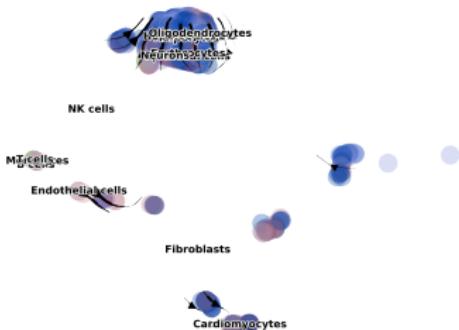
Normal 1

clusters



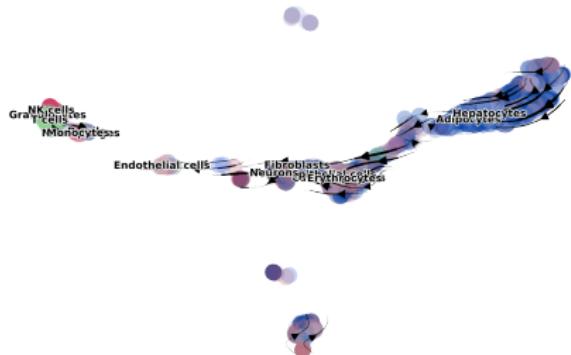
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clusters



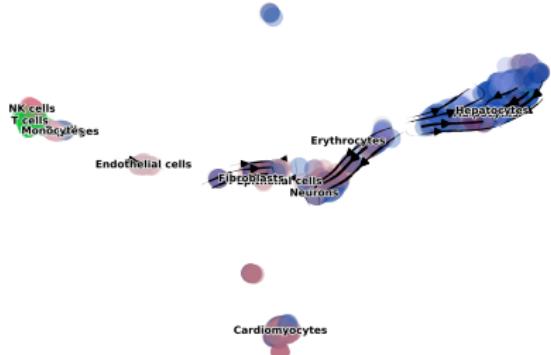
Normal 3

clusters



Normal 4

clusters

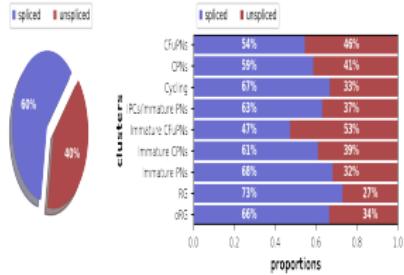


Section 2

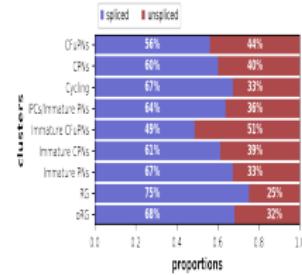
Human data

Looking at the proportion of spliced and unspliced counts

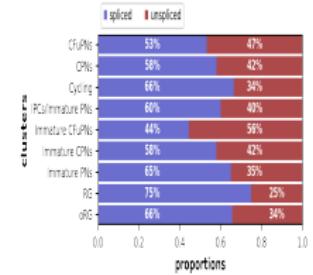
Organoid 1



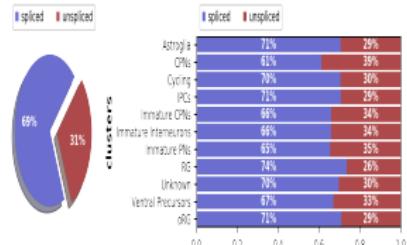
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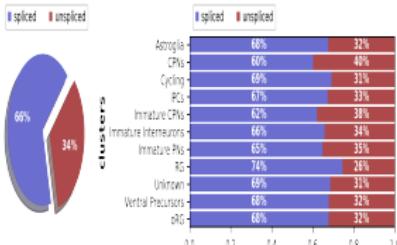
Organoid 3



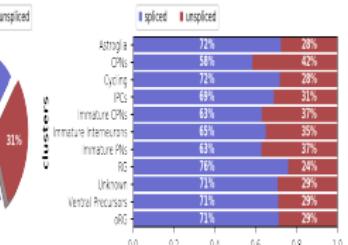
Organoid 16



Organoid 17

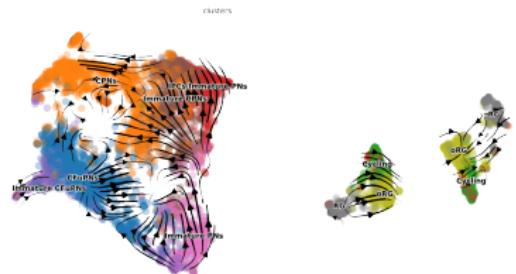


Organoid 18

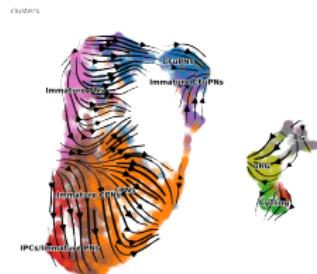


Looking at the RNA velocities - individual samples

Organoid 1



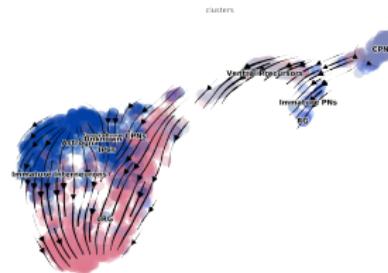
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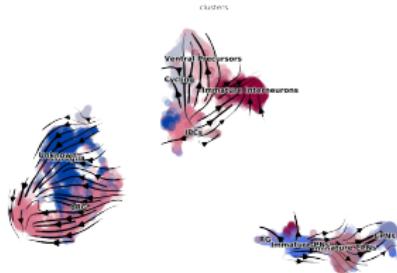
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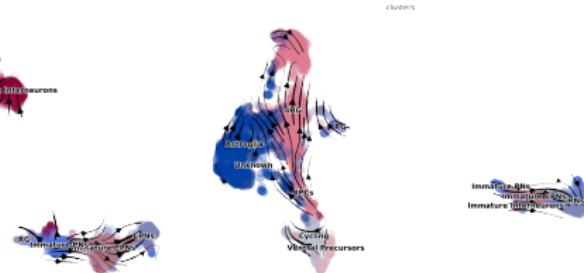
Organoid 16



Organoid 17



Organoid 18



Looking at the RNA velocities - all samples

Organoid 1



Organoid 2



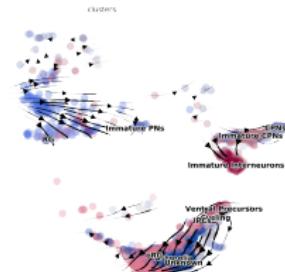
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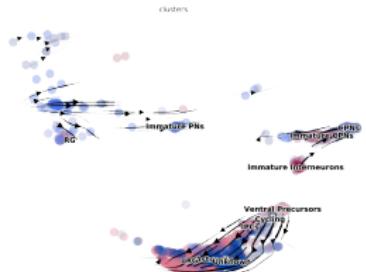
Organoid 16



Organoid 17



Organoid 18



Conclusions from the explorative analysis

- We expected comparable patterns with changing trajectories between experimental conditions
- No clear patterns that were consistent between biological replicates, also arises the question of reproducibility
- Interpretation based on RNA velocity seems not possible

Simulation of data sets from the mouse data

- ① Compute pseudobulk from the four mouse samples and filter for these cell types: **Epithelial cells, Adipocytes and Hepatocytes**
- ② For each of the cell types randomly draw a fixed proportion of genes and cells. Exchange the counts of spliced and unspliced counts for the drawn genes and cells in one experimental condition.
- ③ Differential analysis using eisaR and DEXSeq -> Establishing baseline performance
- ④ Adding multimapping with minnow -> We expect performance of eisaR and DEXSeq to go down, which should improve again with our proposed method

References

- Park, Jihwan, Rojesh Shrestha, Chengxiang Qiu, Ayano Kondo, Shizheng Huang, Max Werth, Mingyao Li, Jonathan Barasch, and Katalin Suszták. 2018. "Single-Cell Transcriptomics of the Mouse Kidney Reveals Potential Cellular Targets of Kidney Disease." *Science* 360 (6390): 758–63.
- Velasco, Silvia, Amanda J Kedaigle, Sean K Simmons, Allison Nash, Marina Rocha, Giorgia Quadrato, Bruna Paulsen, et al. 2019. "Individual Brain Organoids Reproducibly Form Cell Diversity of the Human Cerebral Cortex." *Nature* 570 (7762): 523–27.